INTRODUCTION: Although carpal tunnel syndrome is a fairly common condition, there remains a limited understanding of its molecular pathogenesis. Previous investigators have explored the relationship of graded nerve compression and intraneural microvascular permeability within the blood nerve barrier (1); however, little additional work has been done to evaluate the changes in vascularity associated with chronic nerve compression (CNC). Although it remains unclear if Schwann cells or axons are the principle source of vascular endothelial growth factor (VEGF) within the peripheral nerve, it is certain that the presence of Schwann cells is required to direct angiogenesis (2). We sought to explore the source of VEGF and the changes in spatial and temporal expression of VEGF mRNA and protein in the pathogenesis of chronic nerve compression.

MATERIALS & METHODS:

Surgical Technique. A previously described model of nerve compression was applied to one-month old Sprague rats (200 – 300g) (3,4). A dorsal glutal-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) was atraumatically 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.

Probe Generation. Plasmids for VEGF, FLT-1, and FLK-1 were generous gifts from Dr. Jill Helms at UCSF. VEGF was cloned into a T3 plasmid and was linearized using the Cla I restriction enzyme. The FLK-1 gene was cloned into a T7 plasmid and was linearized using the Xho restriction enzyme. FLT-1 was cloned into a T3 plasmid and linearized using the Not I restriction enzyme. Probes were generated using non-isotopic in vitro transcription with digoxigenin-labelled UTP nucleotides using the Ambion Mega Script Kit.

Preparation of Tissue Samples for ISH and Probe Generation. Following dehydration, nerve samples were embedded in a 1:1 mixture of OCT and Aquamount, cut into 10 um cross sections and mounted on poly-L-lysine coated slides. The nerve cross sections were then fixed again in 4% paraformaldehyde, washed in SSC, and then treated with Proteinase K to permeabilize the tissue. Nerve sections were then treated with hybridization solution consisting of SSC, formamide, Denhardt’s, E. Coli RNA, dextran sulfate, heparin, and single stranded DNA at 1 hour at 42°C. After prehybridization step, nerve sections were incubated overnight at 55°C in hybridization buffer with 0.5µg of RNA probe per nerve section. The following day, these nerve sections were treated with RNase, subsequently rinsed with SSC/EDTA, and then subjected to a stringency wash for 2 hours at 55°C in .1X SSC/1mM EDTA. Nerves were then rinsed again and incubated in blocking solution for 30 minutes before anti-digoxigenin antibody was added. Nerves incubated for 1 hour at 37°C in a 1:1000 dilution of antibody. After another wash, nerves soaked overnight at 4°C in a BCP/NBT colorization solution. Nerves were rinsed a final time and cover-slipped using Kaiser mounting media.

Preparation of Tissue Samples for IHC. Nerve samples were dehydrated and frozen embedded as described above. 10 um nerve sections were then dried and further fixed in 4% PFA for 20 min. The samples were rinsed in PBS and subsequently blocked with 5% dry milk in PBS containing 0.25% Triton-X 100 for one hour. PBS wash was then repeated and samples were incubated overnight in rabbit anti-VEGF IgG (1:500 in PBS). Samples were then washed in PBS (X3) followed by incubation in a fluorescein isothiocyanate (FITC)- conjugated anti-rabbit IgG (1:200 in PBS) for one hour. Nerve sections were again rinsed in PBS and counterstained, in the absence of light, with Vectorstain alkaline phosphatase solution containing DAPI. Sections were visualized under a Zeiss fluorescent microscope using a FITC filter. The receptors for VEGF, FLK-1 and FLT-1 were immunostained in the same manner as VEGF using 1:500 dilutions of the polyclonal antibodies rabbit anti-FLK-1 IgG and rabbit anti-FLT-1 IgG, respectively.

RESULTS: As previously reported (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. Both in-situ hybridization and IHC for VEGF revealed intensely stained Schwann cells, indicating that Schwann cells are the principal source of VEGF upregulation in the peripheral nerve secondary to CNC. At the 2 week and 1 month time points, there was a notable increase in VEGF expression relative to the contralateral normal controls. The presence of VEGF mRNA peaked at the 2 month time point and returned to lower levels at 6 months post-injury. The tyrosine kinase receptors for VEGF, flk-1 and flt-1, also demonstrated a similar pattern of up-regulation in CNC nerve specimens. Functional assessment of vascularity demonstrated a marked increase in the number of blood vessels present in experimental nerves harvested at eight months relative to contralateral control nerves.

DISCUSSION & CONCLUSIONS: Our data shows that Schwann cells are responsible for the increase in VEGF mRNA and protein expression with CNC. Previous reports have shown that Schwann cell number reaches a peak at the 1 month time point (4). As the VEGF protein expression peaks at the 2 month time point, VEGF may not be the principle cause of Schwann cell proliferation. Rather, the Schwann cells may be expressing increased amounts of VEGF secondary to the mechanical stress of CNC. By better understanding the pathogenesis of CNC, we may then begin to improve our treatment through targeted gene therapy.

REFERENCES

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