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## **WORKSHOP 3**

# **Evolving Ideas in Spinal Cord and Peripheral Nerve Injuries: Neuroscience for Musculoskeletal Investigators**

Organizer:  
Ranjan Gupta, MD

Speakers:  
Ranjan Gupta, MD  
Edward Diao, MD  
George Smith, PhD

**Orthopaedic Research Society 2007: Workshop #3**  
Evolving Ideas in Spinal Cord and Peripheral Nerve Injuries.

**Basics of Developmental Neuroscience**

Ranjan Gupta, MD  
Irvine, CA

- I. Neural Induction
  - a. Local Interactions
  - b. Molecular Nature of Neural Induction
  - c. Conservation
    - i. BMP and WNT signaling in neural development
  - d. Control of Neuroblast Segregation
  - e. Notch/Delta/Mash genes
  
- II. Polarity and Segmentation
  - a. Anterior-Posterior Axis and HOX genes
  - b. Dorsal-Ventral Polarity within the neural tube
  - c. Dorsal Neural Tube and Neural Crest
  
- III. Genesis and Migration
  - a. Cell cycle genes- cyclin-dependent kinases (Cdk) and cyclins
  - b. Neural Crest Migration
  - c. Generation of Neurons and Glia
  - d. Molecular Mechanisms of Neuronal Migration
  - e. Post-Mitotic and Adult Neurogenesis
  
- IV. Determination and Differentiation
  - a. Invariant Lineages
  - b. Spatial and Temporal Coordinates of Determination
  - c. Asymmetry
  - d. Gradients and Spatial Organization
  
- V. Axon Growth and Guidance
  - a. Growth Cone
  - b. Dynamic Cytoskeleton
  - c. Directional Cues
  - d. Cell Adhesion
  - e. Repulsive Guidance
  - f. Chemotaxis and Local Gradients including Attraction and Repulsion
  - g. Signal Transduction Pathways
  - h. Axon Regeneration
  
- VI. Neuronal Death
  - a. Trophic support from the synaptic target
  - b. Nerve Growth Factor-target derived growth factor
  - c. Neurotrophin family
  - d. The trk family of neurotrophin receptors
  - e. The p75 neurotrophin receptor
  - f. Caspase family
  - g. BCL-2 control of apoptosis

**Peripheral Nerve Injury**

**Edward Diao, M.D.**  
San Francisco, CA

Slide I:

- Nerve anatomy: Arrangement of afferents and efferents from the peripheral to the spinal cord.
- Arrangement of nerve fascicles, perineurium, and epineurium.

Slide II:

Nerve compression mechanical trauma causes fascicular injury. Schematic diagram of the vicious cycle of acute nerve compression and chronic effects.

Slide III:

Background: The incidence of nerve compression and overuse is dramatically increasing in the general population and in particular, working populations and human activities.

- Human nerve compression studies are limited by inability to longitudinally evaluate multiple time points and scant tissue samples are available to elucidate the injury and inflammation repair events.
- Tissues from compressed human nerves have rarely been available.

Slide IV:

Chronic nerve compression is associated with connective tissue changes which include epineurial fibrosis, perineurial thickening, and ultimately, endoneurial fibrosis.

Slide V:

Inducement of nerve compression in animals can be performed with tourniquets, artery clips, compression clamps, or tubes.

Slide VI:

Nerve compression models previously based on constriction and ligature with limited ability to control changes in microcirculation. Histologic changes include subperineurial edema, demyelination, and epineurial fibrosis with more severe changes occurring at higher pressures.

Slide VII:

Silicone and polyethylene tubes have a body of work regarding nerve compression.

Slide VIII:

What are the studies that look at the relationship between mechanics, nerve compression, neuropathy, and recovery? Mechanical pressure plays a role in the pathophysiology of nerve compression. There are only several relevant animal studies.

Slide IX:

Banding experiments in the rat sciatic nerve were reviewed with multiple different-sized internal diameter tubes. Nerve conduction EMG studies showed changes. Blood-brain barrier integrity was evaluated by horseradish peroxidase, Evans blue albumin and these showed some changes.

Slide X:

McKinnon, Dellon, Hudson, and Hunter, 1995 primary model for chronic nerve compressive with sciatic nerve banding with elastic tubes at the carpal tunnel, four animals, six months of nerve compression.

Slide XI:

Limitation of prior studies.

Slide XII:

Other support for ischemic blood flow etiology Eleven carpal tunnel patients with perioperative laser Doppler (Rempel and Abrahamson, Journal of Orthopedic Research , 2001). Rapid synovial plus FDP tendon explants, varying O<sub>2</sub> tension, 3H-thymidine, 3H-proline, and 35S-sulfate assays performed.

- Hypoxia cause decreased cell proliferation in the synovium, decreased protein synthesis, noncollagen/synovium, and no effect on proteoglycans.
- Novel studies using balloon compression techniques. (Reference: Rydevik, Lundborg, and Bagge, Journal of Bone and Joint Surgery 1991).

Slide XIII:

Rabbit carpal tunnel model was developed to use coronary angioplasty balloon catheters within the carpal canal of the rabbit forepaw to cause pressure that could be reproduced varied in terms of pressure and duration. Dose response analysis of nerve conduction EMG studies correlate pressure to time to develop CTS (Journal of Orthopedic Research, 2005).

- Histologic analysis of compressed nerves using light microscopy show axon dropout and perineural edema with osmium staining.
- Elastin staining show changes in the vessels associated with the median nerve.

Slide XIV:

The rabbit carpal tunnel model on electron microscopy shows changes in myelinated and nonmyelinated axons, Schwann cells, with increased phagocytic cells, and increased collagen associated with nerve compression.

Slide XV:

Peripheral nerves also react to cyclic pressure Slide XVI:

We know that the peripheral nerve can react to controlled tension although stretching beyond 15% causes complete ischemia of the nerve (Lundborg et al. 1973). Peripheral nerve lengthening by controlled isolated distraction has been shown to be effective compared to conventional nerve grafting in a rat model (Reference: Krober et al., 2001).

Slide XVII:

We also know that nerves may react to forces generated in neighboring tissues such as tendon and muscles which certainly occurs in humans. (Reference: et al., 1999, Kurasa et al., 2006). An excellent summary of the nerve compressive response was written by Rempel et al. in JBJS, 1999.

Slide XVIII:

Abbadie and Basbaum in 1998 showed that rat sciatic nerve transection causes sprouting of sympathetic efferent noradrenergic axons and terminals around large cell bodies in the dorsal root ganglion. Also neonatal C-fiber neurotoxin Capsaicin increased sprouting. Moreover, C-fiber function was felt to be affected. The organizer of this workshop, Ranjan Gupta, has performed a body of experiments looking at the compressive neuropathy. His approach has been to look at compression in vivo of the

rat sciatic nerve using banding studies for chronic compression and then extend these both to the mouse sciatic nerve and C58/OLA knockout mice. These animal models have demonstrated changes in nerve conduction velocity as well as changes in behavior. They have shown that nerve compression induces mechanical hyposensitivity but not neuropathic pain.

In terms of in vitro studies, they have used Schwann cell tissue cultures from the sciatic nerve of Sprague-Dawley rats and used parallel plate laminar flow chambers to study the effects of shear stress on the Schwann cells as well as use of a hydrostatic compression chamber to study the effect of compression. They found that there are changes in Schwann cell number and apoptosis occurs with decreased MAG, decreased MPDP, and increased VEGF expression. Furthermore, using sophisticated imaging with nerve teasing techniques, they have found that there are changes in internodal length in response to chronic nerve compression, with decreases in internodal length leading to changes in the electrophysiology of the nerve in terms of conduction quality and conduction velocity. They have also demonstrated demyelination with chronic nerve compression that starts at the paranode and progresses into the internodal areas using the teased nerve fiber techniques. Other cellular events have been explored regarding macrophage recruitment with increase in iNOS protein expression.

In terms of what happens to the specificity of nerve regeneration after injury, there is a body of work by Brushart et al. that has identified the neuroanatomy and cellular behavior of divided axons in nerves that are then introduced into a Y-shaped chamber. The specificity of the sprouting and maturation and the ablation of "incorrect" nerve regeneration by pruning has been documented in a series of elegant experiments.

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## **Spinal Cord Injury**

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The earliest accounts of spinal cord injury were written about 3,700 years ago in Egypt. The papyrus describes two incidences involving either neck fracture or dislocation accompanied by paralysis. The conclusion was “an ailment not to be treated”. This treatment procedure persisted well into the nineteenth century with limited treatment other than traction. Within the last half of the twentieth century development of numerous medical techniques, including spine stabilization, rehabilitation, and bladder management have vastly improved the longevity and quality of life for individuals with spinal cord injury; however, reducing or repairing the resulting spinal cord damage still remains a problem.

Spinal cord injuries usually result from a compressive injury that fractures or dislocates the vertebrae, forcing bone fragments against the spinal cord. The resulting mechanical trauma severs axons, breaks neuronal cell membranes, ruptures blood vessels and induces edema. This swelling further reduces blood flow and oxygen to area surrounding the damage, a process that quickly leads to reduced neuronal activity and spinal shock. Aggressive medical intervention to reduce the swell and partially restore blood flow is thought to be effective in reducing some of the secondary degenerative processes, but not all. A number of factors contribute to secondary degeneration, such as excessive release of excitatory neurotransmitters, free radical production, apoptosis, and inflammation. These process further compromise the health of intact neural tissue and result in progressive tissue degeneration that lasts for weeks. As the injury stabilizes, endogenous repair mechanism become active and astroglia react to re-establish the blood brain barrier. Over time, a “glial scar” and persistent cavities form in the region of severe trauma, remyelination remains incomplete, and cut axons fail to regenerate within an inhibitory environment. Exciting advances in all areas of spinal injury have been made; however, for this workshop I will only examine those that involve axonal regeneration.

It has been know since the early twentieth century that cut axons within the adult spinal cord have the ability to regenerate, but fail to grow and become dystrophic. Studies by Marie Filbin’s laboratory show that during development, growing axons have an abundance of cyclic AMP which dissipates as neurons mature. Adult axons have very little and restoring the levels of cAMP using a combination of dibutyl cAMP and a phosphodiesterase, Rolipram, greatly increases their endogenous regenerative potential to overcome inhibitory factor present in myelin. These inhibitory factors are sequestered in myelin and release upon degeneration. There are three potential inhibitory molecule released from myelin, Nogo, myelin-associated glycoprotein (MAG), and oligodendrocyte myelin glycoprotein (Omgp), all of which induce growth inhibition through the Nogo receptor (NgR) complex. Several laboratories are presently examining potential receptor antagonists to prevent NgR signaling and increase regeneration. In addition to myelin inhibitors, the environment around the injury site is full of other inhibitory molecules, such as developmentally important chemorepulsive agents (semaphorin and Eph/eprin) and reactive astrocyte secreted chondroitin sulfate proteoglycans (CSPGs). Of these, several laboratories have been able to neutralizing the inhibitory nature of the latter and increase axon regeneration within the spinal cord. The method uses the bacterial enzyme, chondroitinase ABC, to digest the glycoaminoglycan residues from the core protein of CSPGs greatly reducing inhibition. Together these study show that axon regeneration can occur by neutralization of the inhibitory environment.

Other studies have also show very good results after application of cells or molecules that increase the growth supportive nature of the environment, such as

Schwann cells, olfactory ensheathing cells, and neurotrophins. There are numerous neurotrophins that can be used to target growth of neuronal subpopulations to better tailor axon regeneration. Several laboratories including mine have demonstrate robust regeneration after overexpression of neurotrophins using viral vector systems, and we have further shown that when used in combination with guidance molecules axon regeneration can be targeted to specific spinal cord lamina. Of all these studies the most impressive to date have used combinations of factors to increase regeneration. Dr. Mary Bunge's laboratory has demonstrated superb regeneration and functional recovery with a combination of Schwann cells, cAMP and Rolipram. The use of such combined treatment regimes will most likely prove to be the most efficacious and have the highest potential for inducing functional recovery after spinal cord injury.