Introduction: Ciliary Neurotrophic Factor (CNTF) is described as a neuronal growth factor produced by axonal and Schwann cells and belongs to the interleukin-6 (IL-6) super family of cytokines. CNTF is presumed to promote the differentiation and survival of a wide range of cell types in the nervous system of mammals. Following inflammatory injury, CNTF mRNA is expressed in the hypothalamus, cortex, muscle, and the liver of injured animals and human patients. The development of spinal cord injury following an acute traumatic event is thought to occur through both primary and secondary pathways. The development and progression of secondary pathways is at least in part mediated via the pro-inflammatory cytokine response to injury, reperfusion calcium flux and the elaboration of superoxide radicals. Clinical studies have suggested that pharmacological therapies may be effective in minimizing the observed outcomes following acute spinal cord injury. To date, no agent has been proven to be totally effective. Encouraging results have been observed for the use of steroids in an acute setting, and steroids show the most promise for clinical benefit at this time. However, a more detailed understanding of the mechanism(s) associated with the clinical improvement achieved by steroid administration is necessary. In this study, the investigators have characterized the local tissue CNTF response at the site of injury to “human dose equivalents” of Methylprednisolone (MP) administration using a rat model for acute spinal cord injury.

Methods: The experimental procedure and animal use protocol was approved by the University of Colorado Animal Care and Use Committee, protocol # 31911396(01)2F. Forty Sprague-Dawley rats (450-475 gm), were divided into control and experimental groups. Experimental animals were treated with a “human dose equivalent” (HDE) of MP 15 and control animals were untreated. The HDE dosage is based on toxicity data from various animal and human animal models and is calculated in man on a MP dose in mg/m^2 using a standard conversion formula.

Experimental animals underwent implantation of a twenty gauge subclavicular catheter in preparation for MP administration. Control and experimental animals received an atramumatic two level lower thoracic laminectomy. Spinal cord injury was achieved using an Allen Weight subclavicular catheter, followed by continuous twenty-three hours of HDE MP (31.5 mg/kg/hr) using a Harvard Pump System (Harvard Multi-Syringe Pump #22 South Natick, MA).

Animals were sacrificed at 0, 8, 24 and 48 hours post injury by lethal intracardiac injection of Pentobarbital. Animals were then dissected free of the vertebral column and placed into fresh ice for 1 hr and then placed into chilled 4% paraformaldehyde for 24 hrs. Following sacrifice, the spinal cords were then dissected free of the vertebral column and placed into fresh 10% neutral buffered formalin for an additional forty-eight hours. The spinal cords were then dissected free of the vertebral column and placed into fresh 10% neutral buffered formalin for an additional forty-eight hours. The spinal cords were embedded in paraffin and serially sectioned for staining.

Spinal cord section from MP treated and control non-MP treated rats were evaluated over a time course 0, 8, 24, and 48 hours post spinal cord injury. Spinal cord sections were cut for hematoxylin & eosin staining 21 days after injury, and for immunohistochemistry (IH) analysis for ciliary neurotrophic factor (CNTF) IH was performed using a primary CNTF antibody (Sigma #4085, anti-CNTF RAT), peroxidase secondary antibody (Sigma EXTRA-1, goat ExtrAvidin kit) using DAB for development. Control slides to assess CNTF antibody specificity for IH evaluation were prepared from CNTF-expressing adenovirus vector (Gift from Lyle Moldawer, PhD University of Florida) transfected RAW 264-7 cells, centrifuged into a pellet after transfection and subsequently prepared for permanent sections. Two separate qualified pathologists reviewed all the slides in a blinded manner using an agreed upon visual scale scoring system for comparison of CNTF IH intensity.

Results: Sections from the immediate post-injury phase (0 hours) of inflammation demonstrated variable and inconclusive IH staining patterns. At 8 hrs, both the MP treated and non-MP treated rats failed to demonstrate CNTF IH positive staining. Positive CNTF staining was observed after 24 hours for non-MP treated rats (Figure 1A) and not observed for MP treated animals (Figure 1B).

Discussion: In this experimental model MP treatment did not alter the onset or degree of necrosis at the zone of injury. Further, MP treatment reduced the development of edema adjacent to the zone of injury. As expected, 0 hour rats displayed a variable and inconsistent staining pattern for CNTF. In the early post injury phase, there is local release of CNTF into the zone of acute inflammation. Eight hours following injury, no CNTF staining was observed, suggesting that there is a potential de novo synthesis of this distal cascade inflammatory cytokine. Interestingly, 24 hours following injury, non-MP treated rats demonstrated positive staining for local CNTF release, which by 48 hours becomes inconsistent and variably expressed. This is in sharp contradiction to MP treated animals that failed to demonstrate positive IH staining for CNTF at time point beyond the initial injury.

These results suggest that MP treatment may have a significant anti-inflammatory effect on the local release of potentially beneficial cytokine mediators ordinarily released in the local area following acute traumatic injury. Proceeding from the hypothesis that CNTF is a beneficial neuronal growth and regeneration factor associated with the inflammatory response to injury, we postulated that a potential negative mechanism for failure to achieve sustained neuronal recovery may be associated with MP induced inhibition of CNTF release at the site of injury.

Histologically, MP treatment reduces the development of severe edema and preserves spinal cord architecture adjacent to the site of injury; however the local elaboration of CNTF a cytokine known to be associated with the regeneration and repair of injured neuronal tissues is impaired.