

# VASCULAR ENDOTHELIAL GROWTH FACTOR - A NOVEL PLAYER IN SCI PAIN

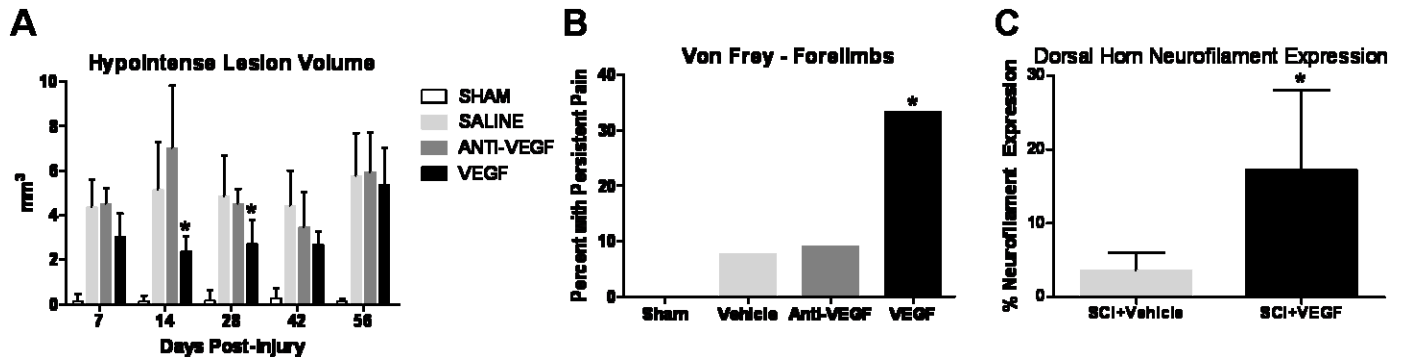
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**Introduction:** Vascular endothelial growth factor (VEGF) is an attractive target for treating spinal cord injury (SCI) because it promotes the growth of new vessels, has neuroprotective effects, and has been shown to induce axonal sprouting. Previous studies have provided conflicting results as to whether treatment with VEGF is beneficial or harmful after SCI; however, preliminary work from our lab suggests that treatment with VEGF may improve injury outcome. The development of neuropathic pain is common in patients with SCI; therefore, we have incorporated tests for mechanical allodynia to supplement our MRI and neurobehavioral assessments. The purpose of these studies was to modulate angiogenic activity, via direct epicenter administration of either VEGF or Anti-VEGF to suppress angiogenic activity, to determine the effect on SCI outcome. Lesion volume was determined by MRI RARE images to evaluate the evolution of the lesion over a period of 56 days and the animals' functional recovery was assessed by a variety of behavioral assays as well as the von Frey filament assay to test for mechanical allodynia.

**Methods:** A total of 60 adult male Sprague-Dawley rats, each weighing between 300 to 350 g, were used in these studies. The rats were assigned to one of four groups: SCI treated with VEGF (n=17), SCI treated with Anti-VEGF (n=15), SCI treated with vehicle control (n=16), or laminectomy only (n=12). All animals underwent surgery under isoflurane anesthesia in which they received a laminectomy; animals in the injured groups received a moderately severe contusion at level T7 using the Infinite Horizon Impactor. The assigned treatment was administered at the time of surgery via direct injection into the site of injury. An 11x35 mm implanted RF coil was positioned above the site of injury and was inductively coupled to an external coil for improved signal-to-noise ratio. Prior to each MR session, a battery of neurobehavioral assays were performed to assess the animals' neurobehavioral condition. The von Frey filament assay was used to evaluate mechanical allodynia and animals were assessed to determine whether persistent chronic pain conditions developed. MRI scans were performed on days 7, 14, 28, 42, and 56 post-injury using a Bruker 7T scanner. Multi-slice RARE images were acquired with a rare factor of 4 and TE<sub>1</sub>/TE<sub>2</sub>/TR of 21.2/63.6/3150 ms. A total of 35 contiguous and interleaved 1 mm thick axial images with a square FOV of 2.62 cm and 256 x 256 image matrix were acquired. The RARE images were inspected for lesions; regions of interest, which included areas of hyper- and hypointensities, were selected using the Image-Pro Plus 5.1 software (Media Cybernetics, Inc., Silver Spring, MD) and lesion volumes were quantified. End point histology was also assessed for markers of axons, myelin, and astrocytes. Differences in lesion volumes, histological measures, as well as behavior measures for all treatment groups and were evaluated using Wilcoxin rank-sum test and multiple comparisons were corrected for using the Bonferroni correction for alpha.

**Results and Discussions:** We have found that acute intraspinal administration of VEGF following contusive SCI results in significant tissue sparing at days 14 and 28, as seen with MRI studies (Figure 1A). A novel finding from our study indicates that there was significant increase in persistent, chronic pain in rats treated with VEGF compared to vehicle controls and shams (Figure 1B). We observed that 33.33% of rats treated with VEGF developed persistent pain compared to 9.09% of Anti-VEGF and 7.69% of vehicle-control rats showed signs of the onset pain. Our results also indicated a direct association between VEGF treatment triggering chronic pain, a significant increase in axons in the dorsal columns and dorsal horns, (Figure 1C) as well as a significant increase in the protein levels of Calcitonin Receptor-Like Receptor (CRLR).



**Figure 1:** A) Acute VEGF treatment appears to lessen the amount of hemorrhage and necrosis compared to both Anti-VEGF and saline treated animals, indicating sparing of tissue. B) However, VEGF treatment also leads to increased incidence of mechanical allodynia. C) VEGF animals had significantly increased numbers of axons in the dorsal horns, possibly due to aberrant axonal sprouting.

**Conclusions:** Acute VEGF treatment following SCI results in tissue sparing, as demonstrated with MRI lesion volume analysis. VEGF treated animals also had a higher incidence of chronic neuropathic pain compared to other treatment groups and shams, and histological analysis indicate that these animals also have increased sprouting of axons into areas associated with the transmission of pain. These findings indicate that treatment with VEGF in the acute phase of SCI results in tissue sparing; however it may also encourage non-specific sprouting of axons into the spinal cord areas associated with pain transmission.

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1. Weirich SD, Cotler HB, Narayana PH, Hazle JD, Jackson EF, Coupe KJ, McDonald CL, Langford LA, Harris JH Jr. Histopathologic correlation of magnetic resonance imaging signal patterns in a spinal cord injury model. *Spine* 1990 Jul;15(7):630-8