

COMBINED VEGF AND ANGIOPOIETIN-1 GENE TRANSFER USING AAV VECTORS AFTER SPINAL CORD INJURY

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Introduction: Traumatic spinal cord injury triggers an immediate disruption of the spinal cord vasculature. This disruption to the spinal cord blood vessels result in secondary damage induced by activating a cascade of cellular and molecular changes, such as cellular excitotoxicity, tissue edema, free-radical production, cytokine and chemokine production, and inflammation. In concert with the secondary damage is the generation of an ischemic environment, inadequate angiogenic response, and the inability to stabilize newly formed vessels. These events may underlie incomplete CNS recovery after injury.

Two key factors that regulate the angiogenic response and vascular stability are vascular endothelial growth factor (VEGF) and angiopoietin-1 (Ang-1). VEGF is a major regulator of angiogenesis serving as a potent stimulator of endothelial cell proliferation, migration, and survival. In addition, VEGF is also known for its neuroprotective effects against ischemic injury. VEGF has the ability to induce profound leakage of vascular endothelial in vitro and adult microvasculature in vivo. It is well established that the increase in vascular permeability underlies the significant role of VEGF in central nervous system (CNS) inflammation. In contrast to VEGF, Ang-1 plays a significant role during vessel remodeling, maturation, and stabilization. Thurston et al., demonstrated that systemic Ang-1 production by adenoviral gene delivery resulted in leakage-resistant vessels induced by VEGF or inflammatory agents.

Methods: The protocol used in this study was reviewed and approved by the Institutional Animal Welfare Committee, and the guidelines provided in NIH Guide for the Care and Use of Laboratory Animals were strictly followed. A total of 30 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 AAV-VEGF (n=6), SCI treated with AAV-Ang-1 (n=6), SCI treated with AAV-VEGF/Ang-1 (n=6), SCI treated with viral control (n=6), and saline only (n=6). 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 at a concentration of 3×10^{12} u/ml. 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. 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 TE₁/TE₂/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. Dynamic contrast imaging (DCE) was performed to determine the integrity of the blood spinal cord barrier. Differences in lesion volumes, DCE, as well as behavior measures for all treatment groups were evaluated using Wilcoxin rank-sum test.

Results and Conclusions: Our data indicates that the combined administration of AAV-VEGF and AAV-Ang-1 results in reduced lesion volume by MRI compared with viral controls. DCE imaging, performed to examine the effect of viral treatment on BSCB permeability, indicated a significant increase in the amount of non-enhancing in AAV-VEGF/Ang-1 treated animals compared with viral controls in the region rostral to the lesion epicenter. The open field locomotor BBB assay indicated a significant improvement in AAV-VEGF/Ang-1 treated animals compared with viral controls. Western analysis of the lesion epicenter indicated a significant increase in viral vectors Ang-1 and VEGF protein expression in the chronic phase of injury. Spinal inflammation was assessed through quantification of microglia, determined by the expression of Iba-1, a calcium-binding protein, infiltrating into the lesion epicenter. Western analysis of Iba-1 indicated no significant difference in microglial recruitment in injured viral-treated versus injured-control subjects, suggesting that viral treatment did not elicit a greater inflammatory response compared with viral control and saline treated groups. This study indicates that viral gene delivery promoting angiogenesis in combination with vessel maturation may be a promising therapeutic candidate for SCI treatment.

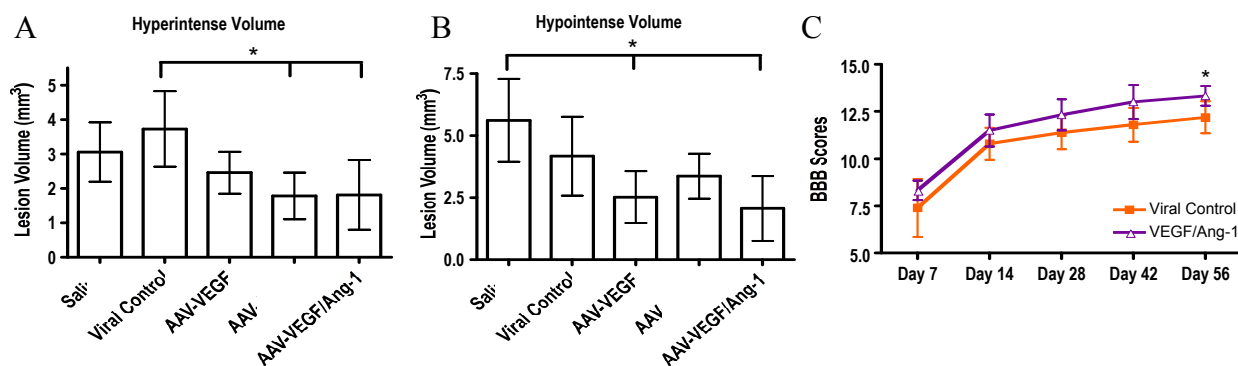


Figure 1: The combination of AAV-VEGF/Ang-1 treatment appears to reduce the hyperintense (A) and hypointense (B) signals at 56 days post-injury. The synergistic treatment of AAV-VEGF and AAV-Ang-1 improves functional recovery based on BBB open field locomotor assay (C).

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