

Vascular Stabilization with Angiopoietin-1 Improves Outcome in Experimental Spinal Cord Injury

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Abstract: The effect of angiopoietin-1 (Ang-1) and adeno-associated virus (AAV) engineered to express Ang-1 on experimental spinal cord injury (SCI) was investigated using longitudinal in vivo MRI, including dynamic contrast enhanced MRI, and neurobehavioral studies. In treated animals, on MRI, improvement in the blood-spinal cord barrier (BSCB) integrity and reduced lesion volume were observed compared to the controls. A concomitant improvement in the neurobehavioral scores was observed in treated animals. These studies suggest that Ang-1 treatment is a promising therapeutic strategy in SCI.

Introduction: Disruption of the spinal cord vasculature triggers the secondary inflammatory response to injury that leads to the chronic deficits accompany spinal cord injury. The disruption of BSCB causes dynamic microvascular instability that has been hypothesized to be a primary event leading to chronic pathology after SCI. Specifically these vascular events include changes in blood flow, intraparenchymal hemorrhage, inflammation, and ischemic. These events may underlie incomplete CNS recovery after injury. One particular agent that has been demonstrated to stabilize disrupted vasculature is angiopoietin-1. Ang-1 plays a critical role in vascular remodeling and maturation into functional blood vessels, along with endothelial cell sprouting, and overexpression of Ang-1 results in leakage resistance in blood vessels. In this study we have investigated the effect of Ang-1 treatment on experimental SCI using longitudinal in vivo dynamic contrast enhanced MRI and high resolution anatomical MRI and behavioral assessment.

Methods: A total of 20 adult male Sprague-Dawley rats, each weighing between 300 to 350 g, were used in these studies. All animals underwent surgery under isoflurane anesthesia in which they received a laminectomy, followed by a moderately severe contusion injury at the T7 level using the Infinite Horizon Impactor. The rats were divided into two groups, with ten in each group. The first group was treated with Ang-1 (1 μ g/ml) and adeno-associated virus (AAV) engineered to express Ang-1 (AAV-Ang-1). The second group which served as the control was treated with vehicle and AAV-LacZ. Ang-1 administration is necessary for immediate vascular stabilization, while the viral delivery provides sustained delivery after about two weeks. The assigned treatment was administered at the time of surgery via direct injection into and around the site of injury at a concentration of 3 $\times 10^{12}$ 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, the Basso-Beattie-Bresnahan (BBB) assay was performed to assess the animals' locomotion. MRI scans were performed on days 3, 28, and 56 post-injury on a Bruker 7T scanner. Multi-slice dual echo RARE images were acquired. Following the anatomical scans, pre-contrast T1-weighted spin echo, axial images were acquired using identical geometry. Then, without moving the animal, a 0.2-mL bolus of Gd was injected in less than 5 sec into the jugular vein via the vascular port. Immediately following the administration of Gd, T1-weighted images were continuously acquired at 30 time points with a temporal resolution of 2 min. The lesion volumes were quantified on T2-weighted images using the Image-Pro Plus 5.1 software (Media Cybernetics, Inc., Silver Spring, MD). DCE-MRI was analyzed using a two compartment model to determine the transfer coefficient Kps, which is a measure of the BSCB integrity. The Differences in lesion volumes, DCE, as well as behavior measures for all treatment groups were evaluated using Student's t-test.

Results: The values of Kps at three different time points are shown in Fig 1 along with the temporal changes in the BBB scores in both groups are summarized in Fig. 1. These results indicate improvement in the barrier integrity with post injury time that is statistically significant compared to the controls by day 56. In Ang-1 treatment resulted in reduced lesion volume by MRI compared with viral controls (data not shown). The open field locomotor BBB assay indicated a significant improvement in Ang-1 treated animals compared with viral controls.

Discussion: In this study we exploited the immediate and short term benefit of Ang-1 and the long term sustained delivery of Ang-1 to stabilize the vasculature in SCI. AAV delivery overcomes the problems with repeated injections of Ang-1. The reduced lesion volume

in Ang-1 treated animals perhaps can be interpreted as neuroprotection. This study indicates that viral gene delivery for promoting vessel maturation may be a promising therapeutic candidate for SCI treatment.

Figure 1: The combination of Ang-1 (1 μ g/ml) and AAV-Ang-1 treatment appears to show trends in decreasing Kps at (A) 72 hrs, (B) 4 wks with significance reached at 8 wks (C) (p=0.013). Open field locomotor assays indicated a significant improvement in Ang-1 treated animals compared to viral controls beginning at 28 days post-injury persisting into the chronic phase of injury.

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