Effect of VEGF Treatment on the Blood-Spinal Cord Barrier Permeability in Experimental Spinal Cord Injury: Dynamic Contrast-Enhanced Magnetic Resonance Imaging

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Introduction

Following mechanical trauma to spinal cord, a series of pathobiological events ensue leading to the so-called "secondary injury". Angiogenesis and disruption of the blood-spinal cord barrier (BSCB) play an important role in the evolution of secondary injury in SCI. Vascular endothelial growth factor (VEGF) is a potent promoter of angiogenesis and leakiness of the BSCB. However, its role in SCI recovery is controversial. Part of this controversy is due to lack of information about the spatial and temporal evolution of the BSCB permeability following administration of VEGF in SCI. Traditionally, the BSCB permeability has been assessed ex vivo, using histological techniques [1]. However, noninvasive in vivo techniques for evaluating the BSCB permeability, such as dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI), are highly desirable [2,3].

Methods

Spinal cord injury

Male Sprague-Dawley rats underwent a controlled moderately severe spinal cord injury at level T7 [4]. A piece of Gelfoam soaked with saline or VEGF was placed on the contusion site immediately after SCI. For improved SNR in MRI, an RF coil was implanted subcutaneously over the injury site without touching the spinal cord. For intravenous delivery of Gd during the DCE-MRI scans, the right jugular vein was cannulated and a vascular port with silicone tubing was implanted.

In vivo magnetic resonance imaging

All MR studies were performed on a 7 Tesla Bruker scanner. On the day of MRI scan, animals were anesthetized with isoflurane and then intubated and mechanically ventilated for the duration of the scan (approximately 3 hours). Silicone tubing was attached to the jugular port with the other end of the tubing attached to a two-way valve. Each of the two ports of the valve was connected to a syringe, one filled with Gd at a concentration of 0.1 mmol/kg and the other with 0.9% saline. Following the acquisition of a tri-pilot scan (for locating the spinal cord) and high resolution anatomical images, pre-contrast T1-weighted spin echo, axial images were acquired (acquisition parameters: TR = 500 ms, TE = 10.4 ms, in-plane resolution = 100 μm, and slice thickness = 1 mm). Then, without moving the animal, a 0.2 mL/kg bolus of Gd was injected in less than 5 seconds 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 minutes, as part of the DCE-MRI scan. Animals underwent DCE-MRI scans during the acute (day 3), subacute (days 7 and 14), and chronic (days 28, 42, and 56) post-SCI time periods. In addition to temporal periods, spatial regions along the length of the spinal cord were defined as follows: caudal (4-8 mm caudal to the injury epicenter), epicenter (≤ 2 mm away from the injury epicenter, including the epicenter slice), and rostral (4-8 mm rostral to the injury epicenter). When the BSCB is compromised, Gd leaks out of the systemic vasculature into the spinal cord, rendering the tissue hyperintense on T1-weighted MRI scans. Areas containing somewhat compromised BSCB but that appear normal on post-contrast T1-weighted images (termed non-enhancing (NE)) are also observed. In these studies, we focused our DCE-MRI analysis on the NE regions since they have been shown to contain important information about SCI-induced disruption of the BSCB [5].

Mathematical model of Gd distribution in the rat spinal cord

For quantification of Gd leakage through the compromised BSCB, a two-compartment model was employed [2]. One compartment represents the systemic circulation (intravascular) and the second compartment represents the extravascular extracellular space (EES) within the spinal cord. The parameter K_{ps} (min^{-1}) represents the transfer rate of Gd from systemic circulation to the EES, and thereby represents BSCB permeability. Data are presented as mean ± standard error of the mean.

Neurobehavioral assay

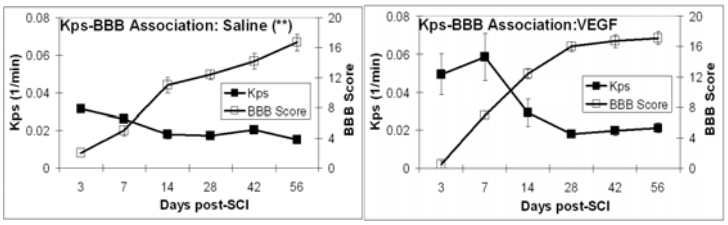
The 21 point open-field locomotor Basso-Beattie-Bresnehan (BBB) assay was performed on the animals immediately prior to their MRI scans.

Results

The BBB score on day 28 post-SCI was significantly greater in the VEGF cohort (16±0.7) compared to the saline cohort (12.4±0.7) ($p<0.0077$, Wilcoxon rank-sum test, corrected $\alpha = 0.0083$). While neurobehavioral recovery was significantly accelerated in the VEGF cohort by day 28 post-SCI, the BBB scores on day 56 post-SCI were not significantly different between the saline (16.7±1.1) and VEGF (16.9±0.7) cohorts ($p<0.92$).

Without consideration for the spatial regions, BSCB permeability (as measured by K_{ps}) significantly decreased over time in both the VEGF and saline cohorts ($p<0.0001$). The value of K_{ps} was greater for VEGF animals compared to saline controls, but only significantly so during the subacute period (0.042±0.0070 compared to 0.022±0.0017, $p<0.01$). When evaluating the change in K_{ps} over both time and space, K_{ps} was significantly greater in the VEGF cohort compared to the saline cohort ($p<0.01$) in the epicenter region, during both the subacute and chronic periods.

The BBB scores were correlated with K_{ps} in the NE areas, using the GEE procedure for a population-averaged model (α corrected for multiple comparisons = 0.0167). The association between K_{ps} and BBB scores was tested for saline and VEGF cohorts separately (see figure to the right). In NE areas only the saline cohort showed a significant overall association ($p<0.001$) between K_{ps} and BBB score



Conclusions

These studies demonstrate that the BSCB permeability was greater at all time points in VEGF-treated animals compared to saline controls, most significantly in the region containing the SCI epicenter. However, a significant temporal reduction in the BSCB permeability was observed in both saline and VEGF cohorts. Our study indicates that acutely administered VEGF hastens neurobehavioral recovery by day 28 post-SCI but that this improvement over saline controls does not persist by day 56 post-SCI. We have demonstrated that non-enhancing areas (NE) on DCE-MRI scans represent areas of compromised BSCB despite their normal-looking appearance. These NE areas may play a significant role in the secondary injury of SCI, and thereby functional outcome. Changes in the BSCB permeability do not appear to be the primary driving factors for VEGF's observed treatment effects in SCI.

References

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