Angiopoietin-1 Reduces Blood-Spinal Cord Barrier Permeability and Lesion Volume in the Acute Phase of Spinal Cord Injury: MRI and Histological Studies

C. B. Patel[1], and P. A. Narayana[1]
[1]Diagnostic and Interventional Imaging, University of Texas Medical School at Houston, Houston, Texas, United States

Introduction

Following mechanical trauma to spinal cord, a series of pathobiological events ensue leading to the so-called "secondary injury". Blood-spinal cord barrier (BSCB) breakdown is an important secondary effect following mechanical trauma to the spinal cord. 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]. Angiopoietin-1 (Ang1) is a protein that has been shown to reduce vascular permeability and stabilize blood vessels [4,5]. The effect of acute administration of Ang1 on BSCB permeability and lesion volume in spinal cord injury (SCI) was quantitatively evaluated using DCE-MRI and high resolution anatomical MRI, respectively. In addition, immunofluorescence histology was performed to validate DCE-MRI results.

Methods

Spinal cord injury

Male Sprague-Dawley rats underwent a controlled moderately severe contusion injury to the spinal cord at level T7 [6]. Rat albumin (vehicle) or Ang1 diluted in vehicle was microinjected into 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 (acquisition parameters: TR = 3200 ms, TE's = 21.2 ms and 63.6 ms, in-plane resolution = 100 μm, slice thickness = 1 mm), 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 phase of injury (2 days post-SCI). Spatial regions along the length of the spinal cord were defined as follows: caudal (3-6 mm caudal to the injury epicenter), epicenter (≤ 2 mm away from the injury epicenter, including the epicenter slice), and rostral (3-6 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 [7]. Lesion volume was quantified based on the high resolution anatomical scans.

Mathematical model of Gd distribution in the rat spinal cord

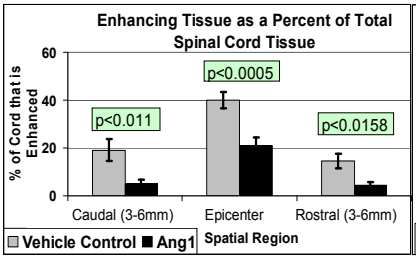
For quantification of Gd leakage through the compromised BSCB, a two-compartment model was employed [2]. One compartment represents the systemic circulation (intravascular) and the second compartment represents the extravascular extracellular space (EES) within the spinal cord. The parameter K_{ps} (min^{-1}) 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.

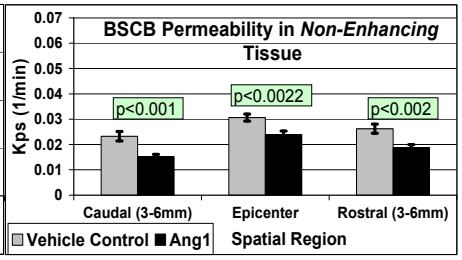
Histology

At the conclusion of the MRI scan, the animals were sacrificed and spinal cords were processed for histology. Albumin is a serum protein that extravasates into the spinal cord after traumatic SCI. Immunofluorescence staining for albumin was performed in order to validate the DCE-MRI studies. The mean fluorescence staining intensity in the ventral gray matter of caudal sections was compared between both groups.

Results

The extent of BSCB compromise was significantly reduced by Ang1, as measured by a reduction in the fraction of enhancing tissue in all three spatial regions (caudal by 3.7-fold: $p<0.011$, epicenter by 1.9-fold: $p<0.0005$, and rostral by 3.2-fold: $p<0.0158$) compared to vehicle control (Wilcoxon rank-sum test, corrected $\alpha = 0.0167$). Ang1 significantly reduced BSCB permeability in NE tissue in all three regions, as measured by K_{ps} (caudal by 1.5-fold: $p<0.001$, epicenter by 1.3-fold: $p<0.0022$, and rostral by 1.4-fold: $p<0.002$) compared to vehicle control (Wilcoxon rank-sum test, corrected $\alpha = 0.0167$).





Ang1 reduced hypointense, hyperintense, and total lesion volume compared to vehicle control, but this reduction was only significant for total lesion volume ($p<0.025$).

Histology revealed a significant reduction in the mean albumin staining signal in Ang1 treated animals compared to vehicle control ($p<0.02$) that is consistent with the DCE-MRI results.

Conclusions

These studies suggest a beneficial role of acutely administered Ang1 in reducing BSCB permeability ($p<0.002$) and reducing lesion volume ($p<0.025$) in the acute phase of SCI. Longitudinal studies are underway to assess the long-term effect of Ang1 on recovery from SCI.

References

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