Longitudinal Changes in Vascular Permeability in Spinal Cord Injury: Dynamic Contrast Enhanced MRI

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Introduction: The integrity of the blood-spinal-cord-barrier (BSCB) is known to be compromised in traumatic spinal cord injury (SCI). However, the role of compromised BSCB in the secondary injury to the cord is not well known. In these studies we have employed dynamic contrast MRI studies to quantitate the *in vivo* serial changes in the BSCB permeability in experimental SCI. In order to understand the role of compromised barrier permeability, these studies were also performed on injured animals that were treated with vascular endothelial growth factor (VEGF) and its antagonistic antibody (anti-VEGF) which are known to modulate the vascular permeability.

Methods: Male Sprague-Dawley rats were used in these studies. They were divided into three groups for treatment with either Ringers solution (controls) or VEGF, or anti-VEGF. Animals were anesthetized with 4% isoflurane, intubated and ventilated with a mixture of 2% isoflurane, 30% oxygen and air. A vascular port was surgically implanted into the left jugular vein for serial administration of gadodiamide (Omniscan). A laminectomy was performed at the T7 level, exposing the spinal cord. A computer-controlled injury device was used to produce a moderately severe contusion injury to the spinal cord. Immediately after injury a small RF coil was placed just above the spinal cord without touching it, and the spinal cord was treated by application of a 3-5 mm piece of Gelfoam (Pharmacia) soaked in either saline, 4 ug of recombinant human VEGF-165 (R & D Systems), or 4 ug Anti-rat VEGF antibody. (R & D Systems). All MRI scans were performed on a Bruker 7 T scanner. Following the acquisition of pre-contrast T1-weighted multi-slice images (TE/TR = 10.4/500 ms, 1 mm thick, 20 slices, FOV = 26.2 mm x 26.2 mm, 128x128 matrix), gadodiamide (287 mg/kg) was injected into the jugular vein through the vascular port and post-contrast T1-weighted images were acquired repetitively for approximately 50 minutes with a temporal resolution of 1.5 minutes. Sequential MRI scans were performed on days 0 (day of injury), 3, 7, and 15. The scans were analyzed by estimating the concentration of Gd in each slice at each time point, using the following formula: [Gd](t) = RIE(t)/(T10 * r1), where [Gd](t) is the concentration of Gd (mM); RIE(t) is the relative intensity enhancement of the ROI; T10 is the T1 prior to injection of Gd; and r1 is the T1 relaxivity of the Gd. RIE(t) was calculated using the formula RIE(t) = (I(t) – I(0))/I(0), where I(t) is the average signal intensity of the pixels in the ROI at time t, and t=0 is time of the pre-injection scan [1]. A two-compartmental pharmacokinetic model of Gd transport was used to describe the transport of Gd across the BSCB into the the extracellular extravascular space (EES), for each 1mm axial slice [2].

In these studies we analyzed regions with diffuse enhancement and mainly confined to grey matter (representing the injury-induced leakiness in the preexisting vessels) and also those regions with focal enhancements that represent neovasculature (based on MRI and BRDU labeling results) [3]. ROIs in the diffuse enhancement regions were determined using the statistical approach described previously [4].

Results and Discussion: Figure 1a shows the temporal changes in the coefficient of transport from the plasma across the BSCB (kps) for the diffusely enhancing regions relative to the value of the day of injury. Data were included only up to 15 days since we could not detect enhancements, using the statistical decision making process [4], after this time. All the animals showed the same pattern of changes in the BSCB permeability: In the VEGF treated animals, regions with diffuse enhancement showed a transient increase in BSCB on day 3, followed by a decrease. The anti-VEGF treatment promoted restoration of the BSCB more than VEGF. This reduced BSCB permeability with anti-VEGF was also associated with improved behavioral measures of locomotion (BBB scores [5]; data not shown).

Revascularization after injury was affected by treatment with VEGF or anti-VEGF, compared to Ringers solution. A typical example of the change in signal intensity of the focal enhancements (neovasculature) with post-Gd administration time in a dynamic contrast enhanced MRI study in one animal at six weeks post-injury is shown in Fig. 1b. The rapid increase in the signal intensity, implying a more permeable BSCB, with VEGF treatment relative to the control and anti-VEGF treatment can be easily appreciated on this figure. As an example, the results of the analysis of these data on a VEGF-treated and anti-VEGF treated animal are shown in Figs. 1c and 1d, respectively. In these figures, data are shown only from day 15, since this is the earliest time point at which the focal enhancements were observed.

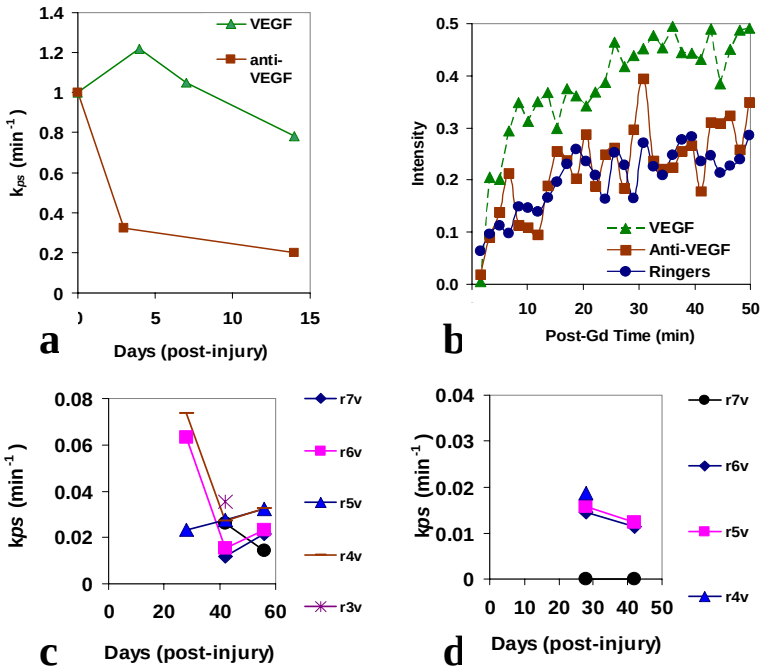

Fig. 1. (a). Temporal changes in transport coefficient (kps) from plasma across the BSCB for the diffusely enhancing regions. (b). Normalized intensity (intensity relative to pre-injection value) of focal enhancements, six weeks post injury, as a function of time since the bolus intravenous injection of Gd. (c). Temporal changes of kps for individual focal enhancements in VEGF-treated (c) and anti-VEGF (d) treated animals. Notation: r3v, r4v, r5v, r6v, r7v, r8v denote vessels that are 3, 4, 5, 6, 7, 8 slices rostral to the epicenter of injury, respectively.

The most striking feature is that the BSCB permeability of the neovasculature is an order of magnitude smaller than the permeability of the existing vessels whose BSCB was compromised by injury. Also the rapid decrease in the barrier permeability with time can be appreciated in this figure. These data also suggest that the permeability is higher in the VEGF treated animals relative to those treated with anti-VEGF.

Conclusions: Serial changes in vascular permeability were determined following traumatic injury to spinal cord. These studies suggest that higher vascular permeability is associated with lower behavioral scores. The data also suggests that anti-VEGF treatment may be beneficial in SCI.

References: [1] Bilgen, M. and Narayana, P.A. Magn. Reson. Med. 46:1099-1106 (2001); [2] Bilgen, M. et al. Magn. Reson. Imaging 20:337-341 (2002); [3] Narayana, P. A. et al. J Neurosci Res (2004) (In press); [4] Bilgen, M. et al., Magn. Reson. Med. 45:614-622 (2001); [5] Basso, D. M. et al. J Neurotrauma 12; 1-21 (1995).

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