Transient blood-brain barrier disruption and changes in brain electrolytes at the edge of the ischemic core

V. E. Yushmanov[1], A. Kharlamov[1], F. E. Boada[2], and S. C. Jones[2,3]

[1]Department of Anesthesiology, Allegheny-Singer Research Institute, Pittsburgh, PA, United States, [2]MR Research Center, Department of Radiology, University of Pittsburgh, Pittsburgh, PA, United States, [3]Departments of Anesthesiology and Neurology, Allegheny-Singer Research Institute, Pittsburgh, PA, United States

INTRODUCTION

The time of the earliest blood-brain barrier (BBB) abnormalities associated with vasogenic edema after stroke has been notoriously hard to assess, as permeability agents only become detectable after enough material has crossed from the vascular space into brain, usually over 6 h. Gd-DTPA, however, has been shown to appear in the sulcal spaces at early times (4 h) after human ischemic stroke (1). The present work documents the extravasation of Gd-DTPA into the cerebrospinal fluid (CSF) directly over the edge of the ischemic region in the rat model of focal ischemic stroke.

METHODS

Sixteen normally fed male Sprague-Dawley rats weighing 340 ± 25 g underwent either permanent MCA transection with bilateral CCA occlusion under halothane (in 30% O_2, 70% N_2O) anesthesia (15 rats) or a sham surgery (1 rat). In 5 rats (4 MCAO and a sham), the BBB permeability was evaluated using 4 to 5 Gd-DTPA (OptiMark) injections with a 1-hour interval and T_1-weighted (T1w) imaging at 1.3-5.7 h quantitating the intensity difference between the pre- and post-Gd-DTPA images (5 and 10 min after injection). Images were obtained on a 3 T GEMS scanner using a dedicated 5-cm-diameter, 5-cm-long birdcage or parallel cosine (PCOS) transmit/receive RF coil. ADC maps were reconstructed from ^{1}H diffusion-weighted (DWI) multislice spin-echo images (*b*-factors of 0, 93, 372, and 837 s/mm^2). The infarct size and location were verified by DWI, T_2-weighted ^{1}H MRI, and by the change in surface reflectivity of ischemic tissue (2). The 40-µm thick coronal brain sections taken every 400 µm at different levels from bregma were digitized and registered to render volumetric reconstructions of the brain. MR and reconstructed 3D images showing reflective changes were aligned and analyzed in AMIDE (3). In a group of 11 rats, brains were sectioned (40 µm) for quantitative K^+ histochemistry (4) calibrated by flame photometry of micropunched samples (2 x 0.5 mm diameter).

RESULTS AND DISCUSSION

Fig. 1 documents the extravasation of Gd-DTPA into the CSF directly over the edge of the ischemic region. A thin line of Gd-DTPA enhancement occurred in the CSF space at the ventral edge of the ischemic region adjacent to the left inferior cerebral vein at 3.6±0.4 h (n=4) and did not occur on the contralateral side and in the sham craniotomy. Fig. 1E shows a sharp rise of the Gd-induced enhancement in the ipsilateral CSF space and ischemic cortex between 2.8-4.4 h after occlusion, but not in the homotopic contralateral CSF space. These initial studies show early, but transient, Gd-DTPA enhancement in the permanent MCAO model that has not been described previously, to our knowledge, in occlusive experimental stroke. The 10 times more Gd-DTPA leakage from the pial circulation than the ischemic cortex might occur because its BBB is less tight: the transendothelial electrical resistance of parenchymal vessels is ~8000 Ω/cm^2, whereas it is ~800 Ω/cm^2 for pial veins (5).

Brain potassium ($[K^+]_{br}$) dropped significantly in all animals at either or both of the dorsal or ventral edges of the ischemic core, similarly to the earlier observation in a suture occlusion model (6) shown in Fig. 2 in a reconstruction from K^+-stained coronal slices. The maximum rate of $[Na^+]_{br}$ increase was also observed at the periphery of the ischemic region (7). These results using Gd-DTPA dynamics, sodium MRI and K^+ analysis point to a common theme of transient BBB disruption at the edge of the ischemic region at 3-4 h after onset of experimental focal cerebral ischemia in the rat. They are supported by other findings: the stability of $[K^+]_{br}$ during the 3 h after ischemic onset (8), a more severe decrease of $[K^+]_{br}$ at the edges of the ischemic region (9), and sulcal enhancement of Gd-DTPA in human stroke (1). All of these events are coordinated in time (3-4 hours after stroke) or place (the edge of ischemic region). We suggest that the transient BBB changes observed with Gd-DTPA together with the maximum decrease in $[K^+]_{br}$ and increase in $[Na^+]_{br}$ at the same peripheral portion of the ischemic region, are related to higher levels of 'trickle' blood flow to the peripheral areas of the ischemic core, and that these events signal the initial onset, and mark the initial position, of vasogenic edema.

CONCLUSIONS

These findings represent the earliest indication of BBB breakdown in the pial circulation, which might correspond to the K^+ and Na^+ imbalances in the ischemic core and signal the onset of vasogenic edema. These data demonstrate the potential of Gd-DTPA enhanced MRI, in conjunction with multiparametric analysis of precisely aligned images (including histologic), to co-localize ischemic regions and BBB changes.

REFERENCES

1. Latour LL et al. *Ann Neurol* 2004, 56: 468-477.
2. Kharlamov A, Kim DK, Jones SC. J Neurosci Methods 2001;111:67-73.
3. Loening AM, Gambhir SS. Mol Imaging 2003;2:131-137.
4. Kharlamov A, Yushmanov VE, Jones SC. *Brain'07*, Osaka, Japan, 2007; Abstr. BP53-7W.
5. Butt AM, Jones HC, Abbott NJ. *J Physiol* 1990, 429: 47-62.
6. Jones SC, Kharlamov A, Yushmanov VE. *Int Stroke Conf 2009*, San Diego, CA, Feb. 18-20, Abstract 1984.
7. Yushmanov VE et al, *Proc ISMRM* 2008, 16: 305.
8. Sick TJ et al. *J Cereb Blood Flow Metab* 1998, 18: 1114-1120.
9. Kato H et al. *Exp Neurol* 1987, 96: 118-126.

SUPPORT: NIH NS30839

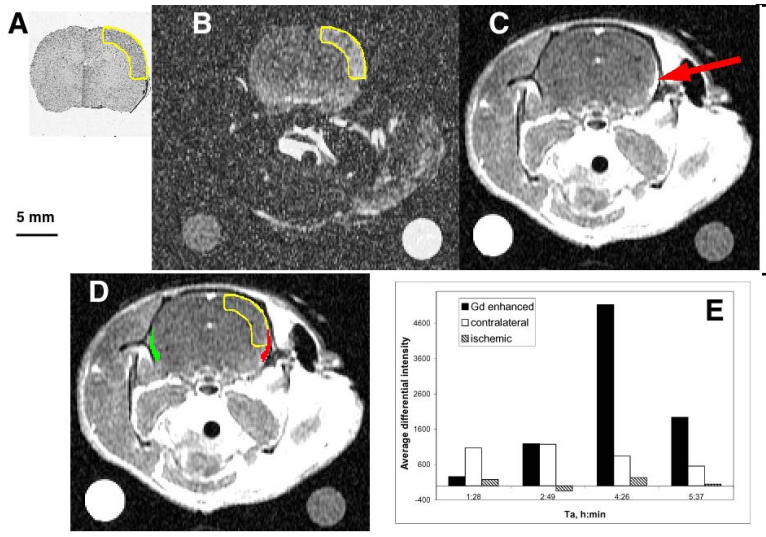

Fig. 1. (A) The ischemic lesion ROI (outlined) identified using the surface reflectivity; (B) a perfect overlap of this ROI with the lesion visible on the DWI scan; (C) the appearance of Gd-DTPA in the CSF space on the ischemic side (arrow) but not on the contralateral side in the T1w image; (D) ROIs for the ischemic region (yellow), the CSF space with maximal enhancement of Gd-DTPA (red), and the contralateral CSF space (green); (E) a sharp rise of the Gd-induced signal enhancement in the ROIs shown in (D) for the ipsilateral CSF space (solid bars) and the ischemic cortex (hatched bars) compared with the homotopic contralateral CSF (white bars). T_a, time after occlusion.

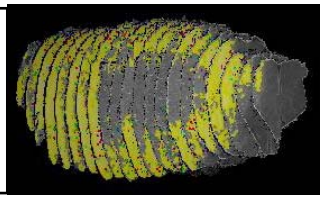

Fig. 2. A 3D brain reconstruction from K^+-stained coronal slices (0.8 mm separation) with the ischemic edge ($[K^+]_{br}$<56 mM, in yellow) surrounding the ischemic core (data from Ref. (6)).