Quantification of blood-brain barrier permeability in the mouse brain in vivo

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Introduction

The blood-brain barrier (BBB) plays a crucial role in protecting the brain from the entry of various neurotoxic or neuroactive agents. Impaired BBB function has been implicated in a variety of diseases including multiple sclerosis and traumatic brain injury for both pathogenesis and disease progression. Therefore, quantitative measurement of BBB permeability is of importance in understanding the disease mechanisms and evaluating potential agents that modify BBB permeability. Quantitative measurement of BBB permeability has been proposed using the multiple time-graphic method or Patlak plot and MRI [2 and refs therein]. Currently, most animal studies of quantitative BBB permeability measurement have been performed on rats or larger animals rather than in mice due to technical challenges. In this study, we report a new approach to quantify BBB permeability in the mouse brain in vivo.

Methods

Three C57BL/6 mice underwent surgery with a controlled cortical impact (CCI) described previously [3]. At day 3 following CCI, an i.v. catheter (P.E. 10 tubing) filled with Gd-DTPA was placed in the jugular vein of the animal. All MR studies were performed at 9.4 T Varian system equipped with a 12 cm gradient insert (40 G/cm, 250 μs) and interfaced to a Varian INOVA console (Varian Inc., CA). A 6-cm diameter Helmholtz volume transmit coil and a 7-mm diameter surface receive coil were used for MR imaging. Anesthesia was induced by 4% isoflurane mixed with 4 L/min air and 1L/min O2 and maintained by 1-1.5% isoflurane. Body temperature was maintained at 37˚C using a circulating hot water pad and a temperature controller (Cole-Palmer, NY). Respiration was monitored via a respiration pillow (SA Instruments, NY). After positioning the animal in the magnet, a T2-weighted MRI (FOV=2 cm, matrix=256x256, TE/TR=30/1500 ms, nt=2, thk=0.5 mm) was measured by a multi-slice spin echo sequence. T1 mapping was performed using a modified Look-Locker multislice sequence to acquire multiple phase encodings per inversion pulse (TR/TE = 4/2 ms, FOV = 2 cm, matrix = 128 x 128, thk = 0.5 mm, flip angle = 20^{o}, 22 inversion times, acquisition time = 8.5 min). A total of 9 sets of high-resolution spin-echo T1-weighted images were acquired before and after a bolus of Gd-DTPA (0.2 mmol/kg) (FOV=2 cm, matrix=256x256, flip=90, TE/TR=10.5/600 ms, nt=2, thk=0.5 mm, acquisition time=2.5min). Changes of T1 values in the brain and the blood were calculated based on the T1 values from the baseline T1 maps and the time course of the T1-weighted signals. The tme course of the contrast agent concentration in the blood was estimated from the regions of interest drawn in the saggital sinus. BBB permeability was calculated based on the Patlak plot method [1,2] pixel-by-pixel basis using a software written in IDL (RSI, CO).

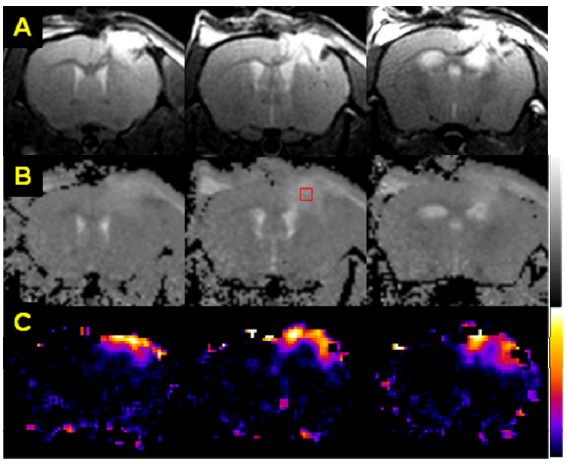

Figure 1. T2-weighted (A), T1 map (B), and BBB permeability map (C) of a mouse with TBI. Gray bar indicates T_1 values of 0 – 2.3 s. The color bar indicates the BBB transfer rate of Gd-DTPA of 0 – 0.018 g/ml/min.

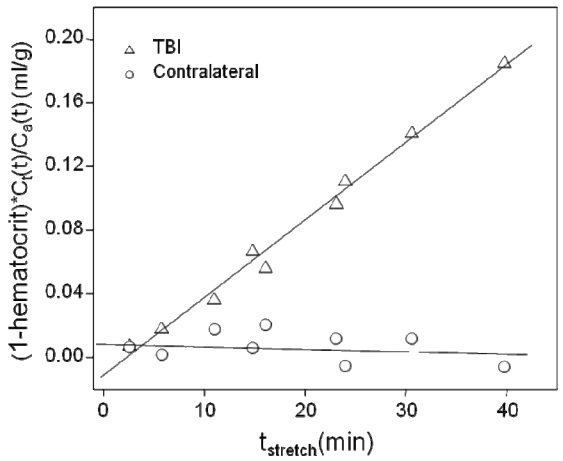

Figure 2. Patlak plot of the regions of interest shown in Fig. 1B and contralateral side of the brain.

Results and Discussion

Figure 1 shows T2-weighted images (A) and T1 maps (B) acquired before Gd-DTPA administration. The cortical areas with tissue damages by CCI are clearly visible as T2 hyper-intensities in the T2 weighted images. Calculated BBB transfer rate maps of Gd-DTPA are shown in Fig. 1C. Increased BBB permeability can be seen in the areas of CCI injury. Figure 2 shows a Patlak plot from the regions of interest placed in the core of the CCI injury and contralateral side of the brain. There is an excellent linear relationship between the tissue concentration of Gd and time (stretched time defined as the ratio of a time integral of the blood Gd concentration and the blood Gd concentration) (R2 = 0.98). Significant entry of Gd into the brain was evident in the injury site whereas there was almost no Gd entry into the un-injured contra-lateral area. The calculated permeability coefficient for the plot was 0.0049 ml/g/min in the injured site.

The current study demonstrates the feasibility of quantitative BBB permeability measurement in the mouse brain in vivo.

References

[1] Patlak et al, *JCBFM* 3:1-7 (1983) [2] Ewing et al., *MRM* 50:283–292 (2003) [3] Bilgen et al., *Neurorehabil Neural Repair* 19:219-26 (2005)

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