Quantitative assessment of blood-brain-barrier permeability by Patlak plots after intraperitoneally administrated gadolinium-DOTA

D. S. Poole[1], J. R. Sikkema[2], A. M. van den Maagdenberg[3], and L. van der Weerd[2,4]

[1]Radiology, Leiden University Medical Centre, Leiden, Netherlands, [2]Radiology, Leiden University Medical Centre, Netherlands, [3]Human Genetics, Leiden University Medical Centre, Netherlands, [4]Anatomy and Embriology, Leiden University Medical Centre, Netherlands

Introduction: Patlak plot analysis of dynamic contrast-enhanced magnetic resonance (DCE-MRI) images enables the estimation of blood-brain barrier leakage following various cerebral traumas. Typically contrast agents are administered intravenously (i.v.) *via* the tail vein, however, i.v. injections often cause scarring, and insertion of permanent canulae in the tail or femoral vein require an invasive procedure and are prone to infection and damage by grooming. Therefore, i.v. administration is less than ideal for repeated longitudinal examinations. As an alternative, we have developed a longitudinal DCE-MRI protocol for mice using intraperitoneal (i.p.) administration of the contrast agent gadolinium-DOTA (CA). Patlak plots[1,2] were employed in order to assess the changes in permeability of the blood-brain barrier (BBB). As a model, mice with unilateral BBB damage resulting from the induction of cortical spreading depression (CSD) were used.

Methods: *Mouse model:* The skull was exposed and a square cranial window (1 mm x 1 mm) ~2 mm lateral and ~3.5 mm posterior right to Bregma was removed. Seven CSD waves were induced in the right hemisphere by applying 30 sec pulses of 1M KCl onto the exposed dura mater. The dynamics of BBB opening were followed by MRI on days 0, 1, 2, 3, and day 9 post-surgery.

MRI scans: In vivo T1W RARE MRI scans were taken of the mouse brain with a Bruker 9.4 T system. For each mouse at each timepoint, a prescan was taken before Gd-DOTA administration, followed by 6 consecutive scans immediately after the i.p. injection. Parameters were: TE = 11.67ms, TR = 870ms, RARE factor = 2, FOV = 20x20mm^2, matrix = 256 x 256, no slices = 22, slice thickness = 0.5mm, 6 averages. Each scan took 11 minutes.

Patlak plots: Patlak plots were constructed by plotting $C_t(t)/C_p(t)$ (ordinate) versus $\int_0^t C_p(\tau)d\tau / C_p(t)$ (abscissa), for various areas in the brain. The slope estimates K_i. The plasma (Cp(t)) and tissue (Ct(t)) concentration of the CA were calculated starting from Equation [1]. The signal intensity (SI) of the regions of interest (ROI), described by Equation [2], were substituted into the mean change in signal intensity (Md) described by Equation [1]. Equation [2] is shown in its simplified form based on a flip angle of 90° and a T2 relaxation time >> TE in our scanning protocol. The resulting formula is shown as Equation [3].

$$Md = \frac{SI(t) - SI(0)}{SI(0)} \quad [1] \qquad SI(t) = PD \times (1 - e^{(-TR \times (R1tissue + R1contrast))}) \quad [2]$$

$$[c] = \frac{-1}{TR \times r_1} \times \ln\left(e^{\frac{-TR}{T_1}} + Md \times (e^{\frac{-TR}{T_1}} - 1) \right) - \frac{1}{T_1 \times r_1} \quad [3]$$

Results: The plasma SI after i.p. administration is shown in Figure 1. BBB leakage was estimated using ROIs for the entire cortex and hippocampus in both the ipsilateral and contralateral hemispheres at ~ -3.0mm Bregma (see Figure 2). The Patlak plots showed linearity up to 72 minutes post intraperitoneal injection of CA (see Figure 3), demonstrating that the CA was transferred unidirectionally from blood to brain, and that the transfer was not limited by the blood flow but by the permeability of the epithelial layer of the blood vessels.

Conclusions: Patlak plots can be calculated with confidence, in MRI studies employing CA administrated i.p.

1. Ewing JR, *et al* (2003) Patlak Plots of Gd-DTPA MRI data yield blood-brain transfer constants concordat with those of ^{14}C-sucrose in areas of blood-brain opening. Magnetic Resonance in Medicine 50: 283-292.

2. Abo-Ramadan U, *et al* (2009) Post-ischemic leakiness of the blood-brain barrier: A quantitative and systematic assessment by Patlak plots. Experimental Neurology 219: 328-333.

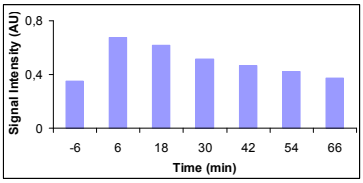

Figure 1: The signal intensities acquired in the plasma before and after i.p. administration of CA at t=0 min.

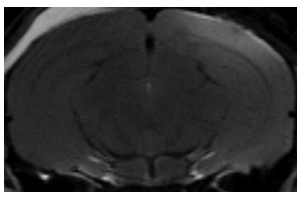

Figure 2: Example of T1W image of the mouse brain positioned at ~ -3.0mm Bregma, 1 day post the CSD induction. The right hemisphere served as induction site for the CSD session.

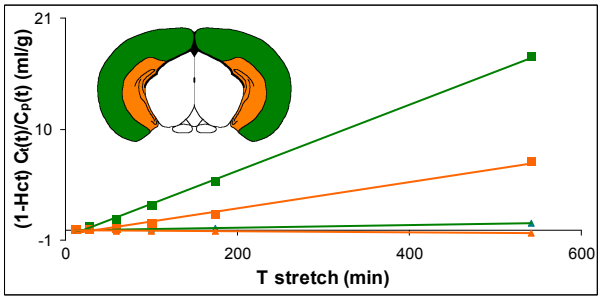

Figure 3: Patlak plots of blood-to-brain transfer rates of the ipsilateral (square) and contralateral (triangle) cortex (green) and hippocampus (orange) at ~ -3.0mm Bregma, of one experiment at one day after CSD.