Blood-Brain Barrier Permeability to Gadolinium and Horseradish Peroxidase after Spontaneous Reperfusion in the Starch Microsphere Model of Ischemia

1N.G. Harris, 2V. Gauden, 3P.A. Fraser, 4S. R. Williams, 4G.J.M. Parker
1Dept. Neurosurgery, University of Cambridge, Cambridge; 2Physiology Division, Kings College London, London; 3Imaging Science & Biomedical Engineering, University of Manchester, Manchester; 4NMR Unit, Institute of Neurology, London, UK.

Purpose
The purpose of this study was to determine the suitability of gadolinium (Gd)-enhanced MRI to quantify blood-brain barrier (BBB) opening that occurs after ischemia-reperfusion. The regional extent of Gd leakage into the extracellular space was compared to horseradish peroxidase (HRP) extravasation in the same animals.

Introduction
Although BBB opening subsequent to ischemia-reperfusion has been studied extensively, the usual methodology is limited to either a single time-point, qualitative assessment of dye permeation or immunohistochemistry, or to quantitative radioisotope measurement. Contrast-enhanced MR imaging enables serial, quantitative permeability measurements to be performed on a regional basis.

Methods
Baseline, single-shot diffusion-trace images were acquired from halothane-anesthetised rats (180 ±17g, n=6) prior to induction of ischemia on the bench by slow injection of starch microspheres (d=40µm) into the right internal carotid artery. The injection continued until cessation of perfusion in the middle-cerebral artery territory, as visualised through the thinned skull. The animal was repositioned in the magnet and diffusion-trace images were acquired to confirm occlusion and to follow the subsequent spontaneous reperfusion that occurs in this model of ischemia. MK parameters were: 40mm²/1st-order view, 128 x 64 data matrix, b values: 0, 1187 s/mm², TR=1 s, 3 contiguous slices; 2mm thick. When brain diffusion measurements were stable, T1 data were acquired using an inversion-recovery, spin-echo, EPI sequence with 7 inversion times: 100, 250, 500, 800, 1100, 1500, 1700ms (NEX=4, TR=1s, 1 slice and all other measurements as before). A nonlinear fit was used to obtain maps of T1, M0 and alpha. The same sequence was run sequentially with two inversion times (100, 1100ms) over 60mins to map the T1 every 48s during a 450µl bolus + 200µl flush injection of Gd-DTPA (0.2mmol/kg) and HRP (0.35mg) into the jugular vein beginning at 70 +5 5mins post occlusion. T1 maps were calculated using the M0 and alpha values obtained previously. Permeability-surface area product (PS) maps were fitted with a compartmental model [1], using Gd plasma concentration time-course data from the literature [2]. Brains were perfusion-fixed, sectioned and reacted with diaminobenzidine to visualise the regional extent of barrier opening to HRP.

Results
One rat showed no ADC changes and no permeability to Gd or any HRP extravasation despite the absence of perfusion. All other rats showed regions of reduced ADC within the hemisphere ipsilateral to the injection. The ADC returned to control values 25-60mins post-injection, although this was highly variable between brain regions. One rat reperfused entirely within 25mins and showed no BBB permeability, presumably due to the short occlusion time. We successfully obtained Gd permeability maps in 6 rats, although it was only possible to calculate the PS product in regions which exhibited a stable rate of Gd uptake (fig 1).

![Figure 1. Plot of T1 values vs time for 3 brain regions following Gd injection (vertical dotted line). Permeability remained low in the contralateral side, but increased in the ipsilateral striatum. Barrier permeability was unstable in the ipsilateral cortex and it was not possible to calculate a PS product map for this region in this rat.](image)

HP staining was observed in only 2 of 6 rats and when present the regional extent of the barrier leak was much smaller than the area of Gd uptake (Fig. 2). The PS value for Gd was increased from intact brain measurements by almost 100-fold; it was 91.7 ±6.1 x10⁻⁵ and 92.0 ±10.8 x 10⁻⁵/min in the cortex and striatum of the reperfused hemisphere, respectively. It is likely that the difference in the regional extent of BBB breakdown observed with the two methods, is due to the different size of the two molecules.

![Figure 2. A diffusion-trace map after occlusion (left), Gd permeability map after reperfusion (middle) and a histological section (right) showing a much smaller area of HRP extravasation than Gd uptake in the same rat (arrow).](image)

Discussion
Contrast enhanced MR imaging has demonstrated BBB breakdown very early after reperfusion, and this may be significant in the development of post-ischemic damage through exposure of the brain to inflammatory mediators. Current methods are inadequate to extract quantitative permeabilities under conditions when the permeability itself is changing (eg the cortex in Fig. 1), and improved models need to be developed in order to capitalize on the information provided by these early measurements of Gd uptake.

References