Molecular Imaging of Endothelial Activation in Acute Stroke Using A Targeted Iron Oxide Nanoparticle Contrast Agent

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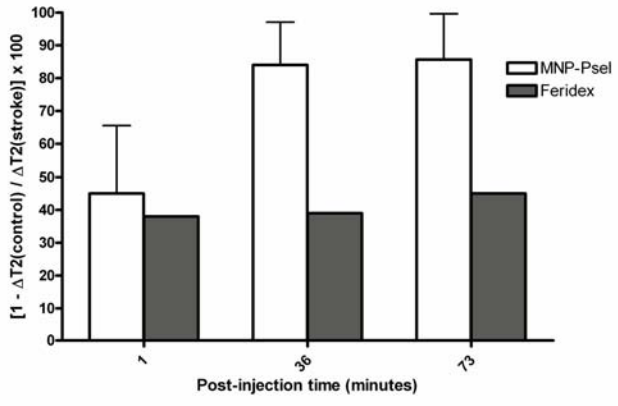
Introduction: Neuroinflammation following acute stroke is believed to play a major role in secondary brain injury and compromised microvascular perfusion. Central to this process is the increased expression of P- and E-selectin in activated endothelium following ischemia/reperfusion. Although the inhibition of selectin adhesion molecules attenuates infarct-related damage, neuroinflammation is a complex process with each tissue, brain region and individual potentially having its own timing and magnitude of response. Because of this inherent complexity, critical aspects of the early evolution of stroke injury are poorly understood. Thus, there is a great need to study the progression of neuroinflammation and endothelial activation non-invasively. Currently MRI can be used to follow the evolution of stroke-related brain injury but it cannot discriminate pathological processes such as inflammation. The aim of this study was to use MRI to visualize endothelial activation in a mouse model of stroke using an iron oxide nanoparticle contrast agent (MNP-Psel) that was modified to bind with high affinity to P-selectin.

Methods: The iron oxide contrast agent MNP-Psel (MW ~ 53000 kDa) used in this study consisted of a 50 nm diameter iron oxide particle coated with aminated dextran to which was covalently attached approximately 1000 molecules of the 15 residue peptide LVSVLDLEPLDAAWL. This peptide was discovered using phage-display technology and has been shown to bind strongly to P-selectin. MNP-Psel was compared to the iron oxide agent Feridex (Berlex), which does not have any binding specificity. As a model for focal cerebral ischemia and reperfusion, the right middle cerebral artery was transiently occluded in male C57 Black 6 mice by an intraluminal occluding silicon-coated nylon suture. The surgical procedure was considered technically adequate if a greater than or equal to 70% reduction in regional cerebral blood flow as measured by laser Doppler flowmetry was observed immediately after placement of the intraluminal suture. MCA occlusion was maintained for 30 minutes after which the intraluminal suture was removed, the proximal ECA was ligated, the surgical wound was closed and the mice were allowed to recover over 24 hours. Normothermia was maintained throughout the procedure and afterwards using an intraabdominal temperature probe with telemetry feedback to a heating pad. Prior to imaging at 24 hours post-reperfusion, the mice were reanesthetized, the intraabdominal temperature probe was surgically removed and a rectal temperature probe was then used for thermoregulation. Mice received either 100 microlitres of diluted Feridex containing 0.0713 mg Fe or 100 microlitres of MNP-Psel containing 0.0713 mg Fe via injection in to the femoral vein. All imaging was done in a 9.4 T/21 cm bore magnet (Magnex, England) equipped with a Bruker console. Animals were positioned in an MR cradle equipped with equipment to monitor respiration, ECG, and temperature. The head of the mouse was positioned in a 3 cm diameter quadrature volume coil. MR scans acquired prior to contrast injection included: multi-echo T2 weighted images (TR=2500ms,TE=10ms between echoes, 24 echoes, number of averages = 2, 128 x 128 matrix, FOV 2 cm x 2cm), T2*-weighted images (TR=1000ms, TE=4ms, 4 echoes, FOV 2 cm x 2 cm, number of averages = 5), and a T1 map (inversion-recovery Snapshot-FLASH imaging scans: TR=3.55s, TE=2.1 ms, increasing T1 delays of 234, 503, 831, 1233, 1751, 2480, 3728, 9226 ms). T1, T2 and T2*-weighted images were repeated immediately after the injection of contrast (e.g. either Feridex or MNP P-sel), and then at 36 and 73 minutes after injection. Infarct regions were defined by areas of increased T2 values at 24 hours post-reperfusion. Post-contrast images were subtracted from pre-contrast images, and the regions with the greatest differences in T1, T2 and T2* were noted. In order to reveal the stroke tissue-specific T2 effects of each contrast agent, the effect of the contrast agents in the contralateral side was subtracted from the effect in the stroke side to remove confounding from any inherent tendency of the contrast agent to accumulate in normal tissue. For this, the following formula was used: [1 – Percent T2 decrease on contralateral side / Percent T2 decrease on stroke side] x 100.

Results: MCA occlusion resulted in similar reductions in cerebral blood flow in all animals. On T1 maps, there was no effect of MNP-Psel or Feridex on T1 values at any post-injection timepoint. On T2 maps, T2 values decreased in both cerebral hemispheres after injection of either MNP-Psel or Feridex. However, in the stroke hemisphere T2 continued to decrease at 36 and 73 minutes indicating accumulation of both MNP-Psel and Feridex. In the contralateral hemisphere T2 also continued to decrease slightly with Feridex but not MNP-Psel, suggesting that the P-selectin-specific contrast agent does not accumulate in the absence of endothelial activation. The T2 decrease attributed to stroke tissue-specific effects increased steadily with MNP-Psel, but not Feridex, during the post-injection period (Figure 1). This indicated that the T2 shortening effect of the P-selectin-specific contrast agent in stroke tissue is less likely to be due to non-specific accumulation of contrast agent. Figure 2 shows an example of the tissue-specific contrast effect of MNP-Psel. Prolonged lowering of T2* values after MNP-Psel was also seen in the stroke hemisphere, but not in the contralateral hemisphere. The T2 and T2* effects of MNP-Psel were observed in both the infarct and peri-infarct regions, suggesting that endothelial activation was present beyond the infarction boundary noted on T2 maps.

Conclusion: In this continuing preliminary study, there is a prolonged shortening of T2 and T2* values within the infarct and peri-infarct region after injection of MNP-Psel. This is in excess of the contrast effect observed after injection of the non-targeted iron oxide contrast agent Feridex. Our results suggest that this T2/T2* effect is due to the binding of MNP-Psel to P-selectin expressed on activated endothelium in the stroke hemisphere. This demonstrates the potential use of targeted iron oxide contrast agents to visualize *in vivo* endothelial activation in acute stroke.

Figure 1. MNP-Psel shows greater stroke tissue-specific contrast effect than Feridex



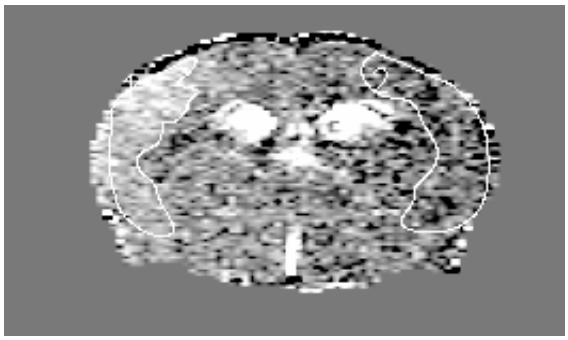


Figure 2. T2 map difference image (precontrast – postcontrast) 36 minutes after MNP-Psel injection; infarct is the bright area on the right side.