Correspondence of Magnetic Resonance Molecular Imaging to Quantitative Determination of E- and P-Selectin Expression in Acute Stroke

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Introduction: Neuroinflammatory processes, such as endothelial activation, are believed to play a key role in brain injury and microvascular compromise in acute stroke and are therefore potential therapeutic targets. Endothelial activation following cerebral ischemia and reperfusion is partly mediated by the expression of the adhesion molecules P- and E-selectin. The development of *in vivo* imaging methods which can monitor and quantify the expression of the selectins would be an important step in assessing their roles in potentially detrimental processes such as leukocyte recruitment. We have previously used magnetic resonance molecular imaging (MRMI) at 9.4T with the selectin-specific contrast agent Gd-DTPA-B(sLe^X)A to obtain a qualitative assessment of selectin expression in a mouse model of focal cerebral ischemia[1]. We hypothesized that MRMI using Gd-DTPA-B(sLe^X)A would demonstrate differences in the accumulation of targeted and non-targeted contrast agent within the ischemic hemisphere in P-selectin knockout mice compared to the wild-type and that these differences would be related to the differential expression of P- and E-selectin between the ischemic and contralateral hemispheres.

Methods: As a model for focal cerebral ischemia and reperfusion, the left middle cerebral artery was transiently occluded in male C57 Black 6 wild-type (n=15) and P-selectin knockout (n=11) 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. Prior to imaging at 24 hours post-reperfusion, the mice were re-anesthetized, the intraabdominal temperature probe was surgically removed and a rectal temperature probe was then used for thermoregulation. Contrast agent – either Gd-DTPA-B(sLe^X)A or the non-targeted agent Gd-DTPA - was injected at 24 hours post-reperfusion. All imaging was done in a 9.4 T/21 cm bore magnet (Magnex, England) equipped with a Bruker console. Mice were positioned in an MR cradle equipped 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) 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. The Gd-DTPA-B(sLe^X)A contrast agent contained a sialyl $Lewis^X$ mimetic derived from the conjugation of 3-[2-(α-D-mannopyranosyloxy)phenyl]phenylacetic acid to DTPA; this compound has previously been reported to have a high affinity for both P- and E-selectin[2]. Following contrast injection, T1 maps were obtained every 11 minutes for 55 minutes. In the cortex of each hemisphere, the pre-post contrast difference in T1 in both the stroke side (ΔT1 Stroke side) and the control side (ΔT1 Control side) was evaluated at each time point. The contrast effect at each time point was evaluated as: ΔT1 Stroke side - ΔT1 Control side.

In order to explain the MRMI findings at a molecular level, E- and P-selectin was quantified in the cortex in the ischemic and contralateral hemispheres using commercially available ELISA methods (R&D Systems) in a separate group of wild-type and knockout mice subjected to transient focal ischemia. Protein determination was done by modified Lowry assay. The amount of E- or P-selectin present in cortical tissue in each hemisphere was normalized to the total amount of protein determined in each tissue sample and expressed as nanograms of P-selectin per milligram of protein, or picograms of E-selectin per milligram of protein.

Results: In wild-type mice, serial T1 maps revealed an accumulation of both the targeted contrast agent Gd-DTPA-B(sLe^X)A and the non-targeted agent Gd-DTPA in the stroke cortex. The magnitude of this contrast effect was similar between Gd-DTPA-B(sLe^X)A and Gd-DTPA. In wild-type mice, quantitative ELISA assessment of E-selectin (Figure A) and P-selectin showed no significant interhemispheric difference between the stroke side and the control side. In knockout mice, there was a significantly increased contrast effect in the stroke cortex with Gd-DTPA-B(sLe^X)A injection that was not observed with Gd-DTPA (Figure B). As expected, P-selectin expression in the knockout mice was nearly absent from all tissue samples. However, the differential interhemispheric E-selectin expression corresponded well to the T1 changes in that E-selectin expression in the stroke cortex was much higher than in the control cortex (Figure A).

Discussion: In this study, interhemispheric T1 changes with Gd-DTPA-B(sLe^X)A in wild-type and P-selectin knockout mice subjected to transient focal cerebral ischemia appeared to reflect the interhemispheric differential expression of E-selectin. In principle, this MRMI method may be developed further to provide a neuroimaging assay of selectin expression which can be performed in the live animal during the acute stroke period. The MRMI findings in this study led to the novel observation that there is increased expression of E-selectin in P-selectin-deficient mice following acute stroke, raising the possibility that expression of one selectin subtype may be upregulated in compensation for a deficiency of other subtypes.

Conclusions: In a mouse model of acute stroke, P-selectin knockout mice demonstrated enhanced targeted contrast accumulation compared to wild-type mice, likely related to increased expression of E-selectin in the ischemic cortex of P-selectin-deficient mice.

References

1. P.A. Barber et al. MR Molecular Imaging of Endothelial Activation in Focal Ischemia. *Ann Neurol* 2004;56:116-120.
2. Y. Fu et al. Synthesis of a Sialyl $Lewis^X$ Mimetic Conjugated with DTPA, Potential Ligand of New Contrast Agents for Medical Imaging. *Eur J Org Chem* 2002;3966-3973.

Figure A. Cortex Expression of E-Selectin: Stroke side-Control side (mean ± SD)

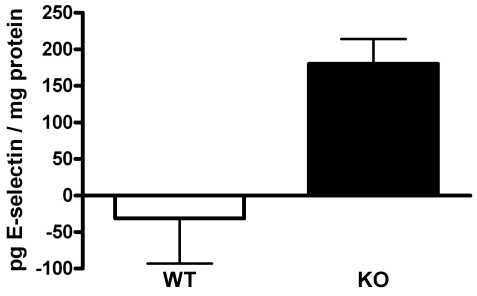

Figure B. ΔT1 Stroke side - ΔT1 Control side After Contrast Injection in P-Selectin KO Mice
(@ p < 0.05, different from corresponding KO-GAD-Cortex timepoint)