IN VITRO MOLECULAR MRI TO DETERMINE CONTRAST SENSITIVITY OF GD DTPA SLEX BINDING TO P SELECTIN

J. Hung King Sang[1], U. Tuor[2,3], R. Muller[4], T. Foniok[2], and P. Barber[5]

[1]Department of Health Sciences, University of Calgary, Calgary, Alberta, Canada, [2]National Research Council of Canada, Calgary, Alberta, Canada, [3]Clinical Neurosciences, University of Calgary, Calgary, Alberta, Canada, [4]Department of General, Organic and Biomedical Chemistry, NMR and Molecular Imaging Laboratory, Université de Mons-Hainaut, Mons, Belgium, [5]Clinical Neuroscience, University of Calgary, Calgary, Alberta, Canada

Introduction

Cerebral ischemia followed by reperfusion induces a harmful inflammatory response in the brain microvasculature in which endothelial expression of P- and E-selectin adhesion molecules are upregulated. Previous magnetic resonance (MR) imaging studies of endothelial activation in the mouse brain post-ischemia have demonstrated rather low contrast sensitivity using a novel contrast probe, Gd-DTPA-B(sLe^x)A. [1]. Thus, prior knowledge of whether a contrast agent binds with sufficient affinity to its intended molecular target could be a valuable tool. The aim of our study was to develop an assay that would allow us to evaluate the MRI contrast sensitivity and the binding affinity of Gd-DTPA B(sLeX)A for P-selectin *in vitro*.

Methods

Phantom Preparation: Soluble P-selectin protein (10 ng/mL) dissolved in coupling buffer (0.1M sodium citrate, 0.05M sodium carbonate, pH 10) was directly bound to aldehyde-activated agarose beads through the formation of covalent secondary amine bonds between P-selectin amino groups and agarose bead aldehyde groups using an AminoLink Plus Immobilization Kit (Pierce). Any remaining active sites on the agarose beads were blocked with 1M Tris-HCl, pH 7.4, and 50mM sodium cyanoborohydride, and unbound P-selectin was removed by washing with 1M NaCl. The immobilized P-selectin was then incubated with either 1mM Gd-DTPA-B(sLe^x)A (n=6) or 1mM Gd-DTPA (n=6) for 2 hours, followed by removal of unbound contrast agent with PBS (0.1M phosphate, 0.15M sodium chloride, pH 7.2) washes. In preliminary experiments, six washes with 100uL aliquots of PBS were determined sufficient to remove the excess contrast agent. Control phantoms (n=3) containing agarose beads bound to P-selectin but no contrast agent were also included for comparison. In a subsequent antagonist experiment, P-selectin conjugated to agarose beads was incubated with an estimated 50 times molar excess of rabbit anti-mouse P-selectin polyclonal antibody (0.13uM) in order to saturate the available P-selectin binding sites. The samples were washed with PBS, and subsequently incubated with either 1mM Gd-DTPA-B(sLe^x)A (n=4) or 1mM Gd-DTPA (n=4), or left without contrast agent (n=2). Again, any unbound contrast agent was cleared by washing the samples with PBS. Imaging Protocol: The phantoms were positioned in a 9.4 Tesla MRI (Bruker System) and scanned using a TRUE_FISP sequence. The scan had a field of view of 2.5 cm^2. matrix of 128x128, TR of 3ms, TE of 1.5 ms, recovery time of 20 s, flip angle of 15^o, slice thickness of 1 mm, inversion pulse of 5 ms with 40 images spaced by 384ms between inversions. Data analysis: Regions of interest (ROIs) of equivalent size were obtained for each phantom, from which mean T1 values and standard deviations were determined for each contrast agent treatment group. Statistical significance was assessed using one-way ANOVA with Tukey's post-hoc test.

Results

Gd-DTPA-B(sLe^x)A incubated with immobilized P-selectin significantly shortened T1 relaxation time relative to the control sample which did not contain any contrast agent ($p < 0.05$, see Fig. 1). Gd-DTPA also reduced T1 compared to the control sample ($p < 0.05$), but the contrast effect was significantly smaller than that observed for Gd-DTPA-B(sLe^x)A ($p < 0.05$, see Fig. 1). The decreases in T1 observed for both Gd-DTPA-B(sLe^x)A and Gd-DTPA treatments were successfully reversed by application of the anti-P-selectin antibody antagonist, as there was no difference in the T1 values between samples incubated with Gd-DTPA-B(sLe^x)A or Gd-DTPA and those without contrast agent ($p > 0.05$, see Fig. 2).

Fig 1. Without Antibody

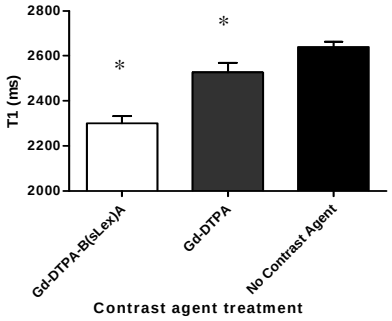
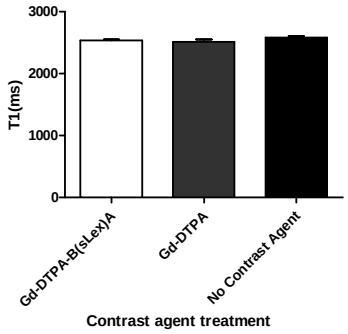


Fig 2. With Antibody



Conclusions

Our results indicate that Gd-DTPA-B(sLe^x)A binds to its P-selectin target *in vitro* and accumulates with a density sufficient to effectively enhance contrast on T1 images. The observed T1 shortening exhibited by the non-targeted Gd-DPTA is likely due to the occurrence of non-selective binding. These conclusions are further supported by blockade of the P-selectin binding sites with anti-P-selectin antibodies, which eliminated the contrast effects previously observed. These results suggest that our assay can be used to semi-quantitatively assess the selective binding affinities of MR contrast agents for their specific molecular targets *in vitro* prior to their use in *in vivo* imaging studies. This assay may be extended to the determination of the dose response of Gd-DTPA-B(sLe^x)A to a given density of P-selectin, and comparisons of binding affinities of various MR contrast probes.

References

1) Barber PA, Foniok T, Kirk D, *et al*. MR molecular imaging of early endothelial activation in focal ischemia. Ann Neurol 2004;56:116-120.