Synthesis of a blood brain barrier (BBB)-permeable MR imaging probe

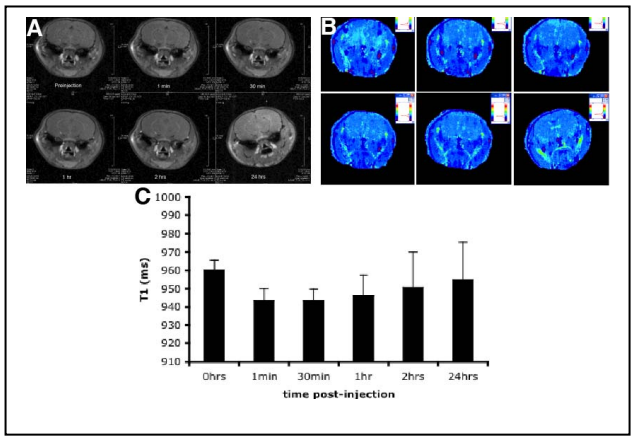
M. Kumar[1], Z. Medarova[1], G. Dai[1], and A. Moore[1]

[1]Molecular Imaging Laboratory, Martinos Center for Biomedical Imaging, Massachusetts General Hospital, Charlestown, Massachusetts, United States

Introduction: The discovery of novel carrier molecules capable of transporting imaging or therapeutic agents across the blood brain barrier (BBB) is an important goal of neurological research. Translocation across the BBB represents a challenging task and thus the delivery is achieved by using invasive direct injection or permeabilization of the BBB. We recently showed that fatty acylated polyarginine peptides (MPAP) have a superior membrane translocation capacity to parental polyarginines (1) and that they constitute a remarkable non-invasive and non-disruptive tool for delivery across the BBB. To explore the potential of MPAP for MR imaging, we modified MPAP to incorporate the paramagnetic contrast agent, Gd-DOTA. The delivery of the contrast agent to the brain could be tracked by magnetic resonance imaging (MRI) and thus would have important clinical implications.

Materials and Methods: MPAP-Gd was synthesized by conventional Fmoc solid phase chemistry by using Rink amide MBHA resin. The final product of C_{14}-β-Ala-$(Arg)_7$-$(Lys\text{-}DOTA\text{-}GD)_2$-$NH_2$ gave optimum yield and was characterized by HPLC and Mass spectroscopy. M/z calcd: - 2729.36 and found: - 2729.32. T1 data for relaxivity determinations were obtained using a standard inversion recovery pulse sequence with delay times ranging from 0.001 to 8000 ms using a 9.4T Bruker horizontal bore scanner (Billerica, MA) at 25°C. To study the toxicity of the compound, 12-wk old balb/c mice were injected intravenously with a range of MPAP-Gd amounts (50 to 200 nmoles) and monitored for viability. Imaging was performed before as well as at 1 min, 30 min, 1 h, 2 hrs, and 24 hrs after intravenous injection of MPAP-Gd (75 nmoles). MRI was performed on a 9.4T Bruker horizontal bore scanner (Billerica, MA) equipped with a home-built RF transmit and receive volume coil and using ParaVision 3.0 Software. T1 maps were acquired using a RARE inversion recovery sequence: TE = 7.413ms, TR = 10,000ms, TI = 0.001, 200, 700, 2,000, and 5,000ms. FOV = 19.2 x 19.2mm^2, spatial resolution = 0.15 x 0.15mm.$pixel^{-1}$, matrix size = 128 x 128, slice thickness = 0.5mm, and a total imaging time of 22min 34sec. For quantitative analysis of T1 relaxation, T1 color-coded maps were constructed using Marevisi 3.5 software (Institute for Biodiagnostics, National Research Council, Canada). The brain was manually segmented and subjected to region-of-interest (ROI) analysis for the determination of T1 relaxation times. For ex vivo studies, the brain was perfused 24 hrs after an intravenous injection of MPAP-Gd. The tissue was homogenized and analyzed by ICP analysis.

Results: The relaxivity of MPAP-Gd shows 9.5 $mM^{-1}s^{-1}$ whereas DOTA-Gd shows 4.2 $mM^{-1}s^{-1}$ at 25°C and 20 MHz. This may be due to the fact that MPAP-Gd has two Gd attached to the sequence and also due to the large molecular weight of the molecule. Our in vivo toxicity experiments demonstrated that the maximum tolerated dose (MTD) of the MPAP-Gd compound following intravenous injection was 75 nmoles. On T1 weighted images, there was a distinctive enhancement of the ventricles after injection of MPAP-Gd (Figure A). This trend was detectable immediately after injection (1min time point), peaked at 30 min, and persisted up to 2 hrs after injection. By 24 hrs after injection, the ventricular enhancement was less apparent. Our T1 inversion recovery sequences mirrored these observations. Immediately after injection of MPAP-Gd, there was a shift towards a shorter T1 (purple) on color-coded T1 maps. By 24 hrs there was an overall shift towards longer T1 values (red), consistent with clearance of the contrast agent (Figure B). Quantitative T1 map analysis revealed a marked shortening of the brain T1 immediately after injection of MPAP-Gd (Figure C). This effect was maximal at 30 min post injection, following which there was a gradual increase in the T1 of the brain, reflective of contrast agent clearance (Figure C). The ICP analysis of brain homogenates from mice injected with Gd-DOTA shows Gd content comparable to the standard sample (without injection of Gd), whereas in mice injected with MPAP-Gd, the Gd content is 20-50 ppb.



Summary: With the increased interest in developing therapeutic agents capable of crossing the blood brain barrier (BBB), there is a clear need for in vivo monitoring of their delivery. Here we report on the synthesis and labeling of the novel MPAP-Gd membrane translocation peptide with the magnetic resonance imaging (MRI) contrast agent gadolinium (Gd), which allows for in vivo MR imaging of the agent's delivery to the brain. Future studies would extend the application of this agent for the combined delivery of therapeutic moieties across the BBB and the monitoring of their delivery.

References:

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