Quantitative Molecular Imaging of Thrombi with Fibrin-Targeted PARACEST Perfluorocarbon Nanoparticles

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Introduction: Fibrin is an abundant component of thrombi and an early marker of ruptured atherosclerotic plaques, which are the major cause of myocardial ischemia and stroke. Identification of fibrin could help detect ruptured plaques and direct therapeutic interventions to prevent or ameliorate the consequences of a heart attack or stroke. Perfluorocarbon nanoparticles (NPs) provide a means to deliver large payloads of imaging agents to the target site and offer the ability to directly quantify binding due to the abundant $^{19}$F signal originating from the particle core. PARACEST (PARAmagnetic Chemical Exchange Saturation Transfer) agents provide an unambiguous MRI signal due to the unique ability to turn the contrast on and off at will. Fibrin-targeted PARACEST nanoparticles were formulated to demonstrate molecular imaging of clots with dual PARACEST and $^{19}$F MRI.

Methods: A water soluble PARACEST chelate, Eu-DOTA-4AMC-benzyl, and lipid-conjugated chelate, Eu-DOTA-4AMC-benzyl-PE, were synthesized. Perfluorocarbon NPs were formulated with Eu-DOTA-4AMC-benzyl-PE (for PARACEST imaging) and biotinylated phospholipids (for subsequent targeting with biotinylated anti-fibrin antibodies). Samples of the water soluble chelate and nanoparticles were prepared with [Eu]=3.33 mM and pH=7. Relaxation times and PARACEST profiles of both samples were acquired at 11.7 T with a 5 s presaturation RF pulse (7 µT) applied at frequencies ranging from -100 to 100ppm. The bound water lifetimes were calculated by fitting the data to the modified Bloch equations. Fibrin clots (n=4) were suspended on sutures in PBS (pH=7), and serially incubated with biotinylated anti-fibrin antibodies, avidin and PARACEST NPs. A quantitation phantom was constructed of NP dilutions ranging from 1.8 to 56.9 nM (pH=7). PARACEST and $^{19}$F images were acquired of the phantom and clots at 11.7 T using a custom-built $^1$H/$^{19}$F coil. PARACEST images were obtained with a 2 s presaturation RF pulse (7 µT) applied on and off the bound water frequency (±51ppm). $^{19}$F imaging of the same slice was performed with identical settings, except 4 times more signal averages. A $^{19}$F reference (28.5 nM NP) was imaged with the clots for quantitation of targeted NP binding. PARACEST contrast-to-noise ratio (CNR) was calculated within regions of interest manually traced for each phantom chamber and the clot surface.

Results: The water soluble PARACEST chelate had similar relaxation properties as the PARACEST nanoparticles, but almost 4 times higher CNR due to a much longer bound water lifetime (NP: 144.7 µs; water soluble chelate: 206.2 µs; Fig. 1). Using the $^{19}$F signal to quantitate nanoparticle concentration, the detection limit (PARACEST CNR=5) of the NP agent in suspension was 4.1 nM. Clots treated with targeted nanoparticles (Fig. 2) showed clear PARACEST enhancement (CNR=17.7 ± 2.8) and $^{19}$F signal (SNR=6.6± 0.8) along the outer clot boundary. The $^{19}$F image intensity corresponded to a nanoparticle concentration on the clot surface of 8.13 ± 1.59 nM and a detection limit of 2.3 nM when PARACEST NPs are bound to a biological target. The lower detection limit is presumably caused by an increase in the bound water lifetime to 185.2 µs (Fig.1) when the particles adhere to a target surface.

Conclusion: We have demonstrated that PARACEST NP can be used for molecular imaging of fibrin with both PARACEST and $^{19}$F MRI. The targeted PARACEST nanoparticles showed a detection limit of 2.3 nM at 11.7T. The improved detection limit for the NP bound to a target versus in suspension may be due to a reduction in the bound water lifetime, which makes the PARACEST exchange kinetics more optimal.