PASADENA: A NOVEL TOOL TO IMAGE ATHEROSCLEROTIC PLAQUE

E. Y. Chekmenev[1,2], P. Bhattacharya[1,2], S. K. Chow[3], A. P. Lin[1,4], D. P. Weitekamp[2], and B. D. Ross[1,4]

[1]MRS, Huntington Medical Research Institutes, Pasadena, CA, United States, [2]Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, CA, United States, [3]Bioengineering, UCSD, San Diego, CA, United States, [4]Rudi Schulte Research Institutes, Santa Barbara, CA, United States

Introduction: The PASADENA (Parahydrogen And Synthesis Allows Dramatically Enchanced Signal Alignment) method offers a promise of increasing the sensitivity of magnetic resonance (MR) over 100,000 times through hyperpolarization subsequent to molecular addition of dihydrogen [1-5]. Magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS) with signal enhancement over 100,000 fold offer multiple advantages including subsecond experimental time, which is especially attractive for cardiac applications. Here, we present a study of a new class of PASADENA agents, which target binding to atherosclerotic plaque by means of a fluorocarbon moiety together with a ^{13}C carboxyl adjacent to a double bond. This class of agents potentially enables the subsecond noninvasive MRI study of cardiac plaque formation with increased spatial resolution and high chemical specificity.

Purpose: Here, we identify lipid-targeted atherosclerotic plaque binding molecules employing a binding assay to dimyristoylphosphatidylcholine (DMPC) lipid membranes by ^{19}F solid-state nuclear magnetic resonance spectroscopy to satisfy the following requirements: (i) PASADENA moiety with C=C double bond for parahydrogen molecular addition, (ii) solubility in aqueous buffers, and (iii) high affinity for atherosclerotic plaque.

Methods: We utilized the acrylate moiety for PASADENA, which has been shown very successful in *in vitro* and *in vivo* application [2]. Various hydrofluorocarbon moieties were inserted as the corresponding arcylate esters. Binding was detected by ^{19}F NMR spectroscopy which provides high sensitivity similar to ^{1}H. In addition, the fluorinated methyl group has a distinct ^{19}F chemical shift. When the fluorinated molecule binds the lipid membrane mimicking atherosclerotic plaque, the fluoromethyl resonance shifts by up to few ppm and becomes broader due to the powder distribution of chemical shift anisotropy typical for partially restricted motion (Fig.1). Binding assays were performed in Bruker Avance data acquisition system at 4.7T with NMR probe tuned to ^{19}F resonant frequency. In the binding assay experiments, NMR peaks were integrated to yield mass and concentration distributions in aqueous solution in contact with DMPC membranes.

Results: Out of a dozen molecular agents screened, we find that 2,2,3,3-tetrafluoro-propyl acrylate (TFPA) combines high solubility (~20 mM) in aqueous buffers at physiological pH, while retaining high binding affinity to the lipid membrane. A kinetics experiment demonstrated that this class of fluorocarbon acrylates binds to the lipid membrane within the first minute of mixing.

To determine the binding affinity, 60 mg of DMPC membranes in 1 mL of 5 mM phosphate buffer were added to 10 mg of TFPA in 5 ml of 5 mM phosphate buffer at 45°C. TFPA binds to DMPC bilayers in a 5:1 molar distribution ratio corresponding to ~5% by weight TFPA enrichment of DMPC membranes (Fig. 1). Fully 33% of all TFPA molecules partitioned into the lipid membrane.

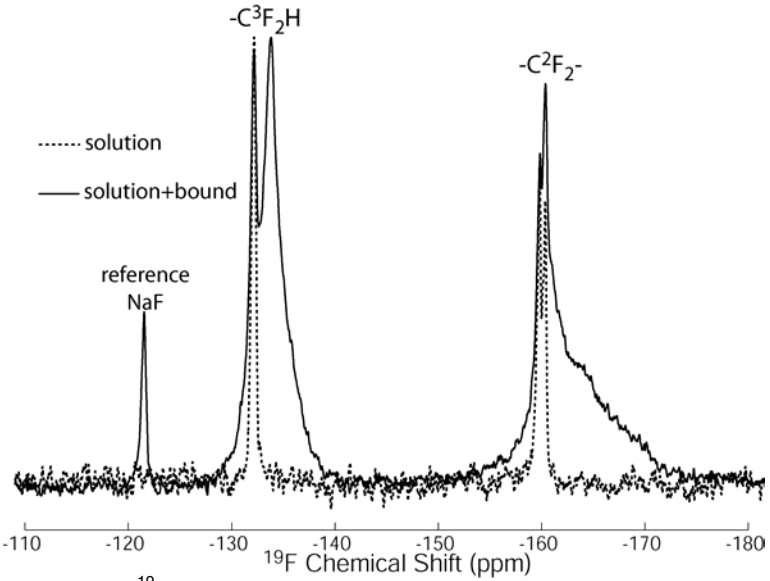

Figure 1. ^{19}F static solid state NMR spectroscopy. Solid line corresponds to the binding experiment of TFPA to DMPC lipid membrane while dashed trace corresponds to the spectrum recorded from 20 mM TFPA solution in water. 25 mM NaF resonance at -121.5 ppm is used as an internal chemical shift reference.

Conclusion: Our results demonstrated efficient and rapid binding of TFPA to a lipid membrane mimicking atherosclerotic plaque. Together with the high aqueous solubility, this shows an excellent prospect for employing this class of molecules for *in vivo* hyperpolarized studies on atherosclerotic plaque formation. Experiments with the hyperpolarized ^{13}C spectroscopy and imaging are underway in our laboratory.

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