Introduction: A promising myeloperoxidase-sensing MRI agent, bis-5-hydroxytryptamidine-diethylenetriamine-pentaacetic acid gadolinium [bis-5HT-DTPA(Gd)] has been recently used for magnetic resonance imaging of in vivo myeloperoxidase (MPO) activity. In the presence of MPO, bis-5HT-DTPA(Gd) can be radicalized to form oligomers and bind to matrix proteins, resulting in increased spin-lattice relaxation rate and prolonged tissue retention [1,2,3]. We recently reported that bis-5HT-DTPA(Gd) allows in vivo targeting of MPO and identifies active inflammation in experimental atherosclerosis [4]. In this study, we measured water proton T1 nuclear magnetic relaxation dispersion (NMRD) profiles for bis-5HT-DTPA(Gd) solutions as a function of temperature in the presence and absence of human MPO plus glucose oxidase (MPO+GO). In addition, we studied the NMRD profiles of tissue excised from rabbit aortas 2 hours after injection of bis-5HT-DTPA(Gd).

Methods: Solutions containing 0.5mM bis-5HT-DTPA(Gd) in PBS with or without MPO+GO were prepared for NMRD measurements. Aortic specimens were excised from 4 cholesterol-fed rabbits (17 months of feeding) and 4 normal diet rabbits euthanized 2 hours after intravenous injection of 0.2 mmol/kg bis-5HT-DTPA(Gd), as well as 4 cholesterol-fed animals and 4 control animals without infusion of the MPO-sensitive imaging agent. A Spinmaster-FFC 2000 (Stelar s.r.l., Mede, Italy) fast field cycling NMR relaxometer was used to obtain 16-point T1 relaxation curves at multiple magnetic field strengths covering the range from 2.5 x10^-4 T to 0.93 T (corresponding to proton Larmor frequencies of 0.01 MHz to 40 MHz), with measurements repeated at four sample temperatures (5 oC, 15oC, 25oC and 35oC). Relaxation curves were fitted by non-linear least squares, and T1 values were extracted from the best fits. NMRD profiles showing R1 (=1/T1) vs 1H frequency were computed for each condition.

Results: Fig. 1 shows the NMRD profiles of bis-5HT-DTPA(Gd) in aqueous PBS solution at 5oC, 15oC, 25oC and 35oC in the absence (left) and presence (right) of MPO+GO. In the absence of MPO+GO, the relaxivity of the agent slightly decreases with increase in temperatures and drops gradually with increasing field strength above 1MHz. When MPO and GO were added to the solutions, bis-5HT-DTPA(Gd) shows a 130-200 % increase in relaxivity over the entire range of magnetic fields. The NMRD curves exhibit a positive temperature dependence with highest relaxivity at 35oC and show a peak relaxivity at approximately 28MHz. Fig. 2 shows the NMRD profiles of aortic specimens, comparing 2 hour post-bis-5HT-DTPA(Gd) injected vs non-injected tissues, and showing atherosclerotic plaque (left) with control tissue (right). The plaque group exhibits substantial bis-5HT-DTPA(Gd)-induced relaxivity enhancement at all field strengths, while aorta specimens from the normal-chow-diet control group did not show significant changes in relaxivity.

Discussion: Recent studies have shown that MPO mediated oligomerization of bis-5HT-DTPA(Gd) results in substantial increases in T1-weighted MR signal intensity. NMRD profiles of bis-5HT-DTPA(Gd) indicate significant relaxivity enhancement over the entire range of magnetic fields. The relaxivity peak at around 28MHz is likely due to an increase in the rotational correlation time of the activated larger agent. With the MPO-mediated relaxivity enhancement increasing with temperature up to physiological body temperatures, this agent is well suited for in vivo assessment of MPO activity. Consistent with our in vivo MRI results [4], the NMRD profiles of atherosclerotic aorta showed relaxivity enhancement, which provides further evidence confirming the agent’s ability to target MPO activity in atherosclerotic plaques in vivo.

References:

Fig. 1. NMRD profiles of bis-5HT-DTPA(Gd) at 5oC, 15oC, 25oC and 35oC. Compared with absence of MPO (left), adding MPO+GO significant enhances the relaxivity over the entire range of magnetic fields. The NMRD profile exhibits a temperature dependence with highest relaxivity after activation at 35oC, and a peak around 28MHz.

Fig. 2. NMRD profiles of aorta specimens at 35oC. Atherosclerotic group (left) exhibits relaxivity enhancement at all tested field strengths upon injection of bis-5HT-DTPA(Gd). Normal-chow group (right) didn’t show significant change of relaxivity after injection of bis-5HT-DTPA(Gd).