Micro-MRI and Fluorescence Imaging of Myeloperoxidase Activity in Human Brain Vascular Pathology

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Target Audience: Physicians and researchers interested in molecular imaging, contrast agents, and micro-MRI.

Purpose: To investigate the feasibility of myeloperoxidase (MPO) activity imaging in human brain aneurysm samples using high-resolution fluorescence and micro-MR imaging using novel substrates.

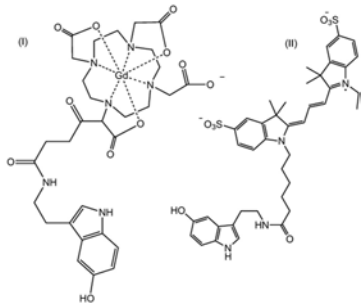

Fig. 1. Chemical formulas of MPO substrates used in this report. I-paramagnetic substrate DOTAGA(Gd)-5HT, Gd salt; II – fluorescent substrate Cy3-5HT (λex/λem=550/565 nm).

Methods: Samples of human saccular brain aneurysm clippings were obtained under an approved UMMS IRB protocol with patient consent (23 aneurysms n=19 subjects). MPO activity was determined in tissue homogenates using a kinetic fluorescence assay. Using PHASES model the normalized activity of MPO was correlated with 5-year aneurysm rupture risk (ARR) in patients. Gd(III) salt of mono-5-hydroxytryptamide of 1,4,7,10-Tetraazacyclododecane-1-glutaric-4,7,10-triacetic acid (5HT-DOTAGA) (Fig. 1(I) was synthesized by reacting tBu-DOTAGA monocarboxylic acid with 5-HT and carbodiimide, followed by TFA deprotection and chelation of Gd(III). Fluorescent substrate (Fig. 1(II), was synthesized by reacting 5HT with Cy3-NHS ester. Relaxivity changes of 0.1-0.5 mM (I) in DPBS in the presence of 1.5 iU MPO were recorded by using Bruker Minispec (0.47T, 37°C). MPO activity in frozen aneurysm sections (8 μm and 50 μm thick) was detected by using 0.5mM substrate II in the presence of 1 mM H_2O_2. Thick 50 μm sections were with 1 mM solution of substrate I, 1 mM H_2O_2 for 2 h. MRI examination on direct tissue slide sections was performed with a set of homebuilt histological coils tuned to operate in 7T μMRI system equipped with an actively shielded gradient coil insert 750-mT/m gradient strength, 100-μs rise time). Depending on the sample (40-μm to 100-μm) and the histology coil used, the MRI in-plane spatial resolution ranged from 57-μm to 100-μm acquired within 6- to 8 h. Pulse sequences used: 2D T1w-GE with TE/TR 3.2/100ms; 2D multi-echo T2*w-GE TE/ES/TR 4.9/4.2/100ms.

Results: Preliminary analysis of MPO [1] and associated MPO activity in the human brain aneurysm tissue suggested positive correlation between normalized MPO enzymatic activity found in the tissue and the increased risk of developing a rupture within a 5-year period. MPO activity also correlated with traditional anti-MPO immunohistochemistry of human samples: aneurysms negative for MPO had approximately 4-fold lower activity than unruptured aneurysms that stained positive with anti-MPO mAb (200.5 iU-MPO/mg). To perform imaging of MPO activity we synthesized paramagnetic and fluorescent reducing substrates for μMRI and microscopy, respectively. With substrate I we observed an increase in molar relaxivity of Gd (r1) as a result of MPO-driven catalysis in the presence of DOTAGA(Gd)-5HT; r1 in the reaction mixture increased from 3.8 to 8.6 $[mM \cdot s]^{-1}$ (i.e. 2.3 fold at 0.47T). Red fluorescent substrate II resulted in localized adventitial staining of the ruptured aneurysm section (Fig. 2) as well as perivascular areas within the lumen of the unruptured (UR) samples that were also subjected to μMRI imaging [2] after incubating of matching parallel thick sections in the presence of 0.5 mM DOTAGA(Gd)-5HT and H_2O_2. The resulting highly detailed MR images (57-μm in-plane resolution) depicted in Fig. 2 (center) and acquired in less than 8 h revealed the presence of high focal T1w-enhancement corresponding to the blood vessel wall (R) and the plaque (UR) areas of matching sections.

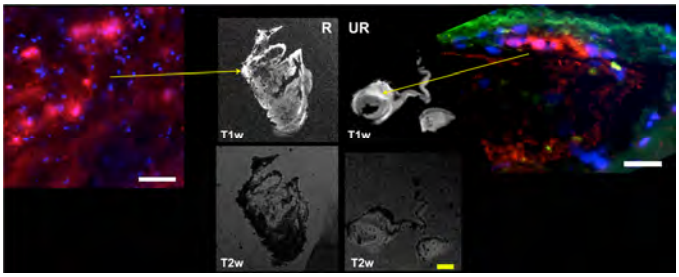

Fig 2. Micro-MR imaging at 7T (T1 and T2 weighted, center) and matching fluorescence images of MPO activity. The μMRI images were obtained after incubating tissue sections in the presence of 0.5 mM paramagnetic substrate (I) / 1 mM H_2O_2 applied to 50μm thick sections of ruptured (R) and unruptured (UR) human brain aneurysm tissue sections. Red fluorescent staining of MPO+ cells was due to the detection of MPO in the presence of II/1 mM H_2O_2. Blue – DAPI (nuclei), Green- collagen autofluorescence. White bars – 100 μm., yellow bar – 1 mm.

Discussion: In humans, MPO is engaged in three roles: 1) bactericidal; 2) stimulatory due in TNF-alpha release by monocytes/macrophages; 3) as an agonist of CD11b promoting endothelial adhesion of leukocytes. Therefore, MPO activity is directly responsible for further activation of cellular inflammatory signaling cascades in vascular disease. The development of clinically acceptable imaging probe for detection of MPO activity would greatly assist in performing differential diagnosis in patients facing potential risk of aneurysm rupture. We previously performed the initial assessment of a potential link between the instability of brain aneurysms and vascular wall inflammation using MPO as a biomarker [1] and performed imaging of MPO activity in animal models [3]. Here we report the use of micro-MRI for detecting MPO activity in human samples as a strategy of validating the specificity of a novel paramagnetic probe DOTAGA(Gd)-5HT by using parallel fluorescence imaging. In contrast to previously used bis-tryptamides of DTPA [4], DOTAGA(Gd)-based MPO substrate showed better solubility and a higher enzyme–mediated molar relaxivity increase due to the lower relaxivity of Gd in a chelated state and lower binding to plasma proteins.

Conclusion: DOTAGA-based paramagnetic MPO substrate is a promising candidate for further clinical translation due to high stability and enzyme-induced r1 increase. High-resolution micro-MRI of human histology samples enable testing of novel MR enzyme-specific probes *in situ*.

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References: 1. Gounis MJ, *et al.,* Stroke; 45:1474-77, 2014; [2] Hoang DM, *et al.,* Magn Reson Med. 71:1932-43, 2014; [3] Gounis MJ at al. AJNR, 2014 doi: 10.3174/ajnr.A4135; [4] Chen JW, et al. Radiology 240:473-81, 2006.