

Molecular imaging of inflammation in a cerebrovascular aneurysm model.

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Introduction It is estimated that more than 10 million people in the US have a brain aneurysm; however, only 30,000 present annually with a brain bleeding from a ruptured aneurysm. Of the patients with ruptured aneurysms, approximately 50% die within 30 days of the rupture. Therefore, there is a strong need in improving the ability to identify aneurysm patients that would benefit from preventative interventional or surgical procedures. There is strong evidence suggesting that the development of cerebrovascular aneurysms and the progression from stable to unstable aneurysm involves local inflammation. The extent of the inflammation is significantly higher in ruptured vs. unruptured aneurysms [1] with neutrophil adhesion molecule CD11b being a marker of ruptured aneurysm. Neutrophils are terminally differentiated granulocytes that secrete large numbers of myeloperoxidase (MPO)-rich granules in inflammatory sites. We previously developed a strategy for imaging of peroxidase activity *in vitro* [2] and *in vivo* [3] using the effect of enzyme-mediated MR signal amplification (MRamp) [4]. The developed approach is based on polymerization and retention of small paramagnetic molecules as a result of the local expression of oxidoreductase activity. We used a 5-hydroxytryptamide (5-HT) based substrate designed for imaging myeloperoxidase (MPO) [5,6] to perform initial testing of the 3T MRI molecular imaging approach in rabbit models of aneurysm (1). We further report the optimization of rabbit model and imaging parameters for myeloperoxidase imaging.

Methods MPO substrate was synthesized and purified as described in [6]. To generate model vascular aneurysms we used elastase-induced modification of vascular wall *in situ*. This procedure results in saccular aneurysms at the base of right carotid artery. A total of 12 NZ white rabbits were used. After 21 days of aneurysmal maturation we used two alternative procedures for inducing vascular intramural inflammation: 1) Under 3-D roadmap, a hypotube was advanced through a microcatheter into the dome of the aneurysm and a fixed volume of 2 μ g of E.coli lipopolysaccharide (LPS) in 0.1 ml saline was injected into the vascular wall. The control animals received a sterile saline injection in a sham procedure; 2) 2 μ g of LPS was injected through a microcatheter placed within the aneurysm under temporary balloon occlusion sealing the neck of the aneurysm from blood flow (Fig 1). Following incubation of the LPS for 5 minutes, the material was aspirated through the microcatheter and the balloon deflated. The control group received the same procedure with LPS replaced by sterile saline. 48 h after the injection, animals were subjected to 3T MRI with ECG/respiratory gating using 3D T1W-FFE sequence and double-inversion recovery STIR technique (TSE/SE TR/TE=822/9.4 ms FA 90, NEX=8, 1 mm slice) to obtain axial images of the aneurysm. Animals then received an intra-arterial injection of di-5-HT-GdDTPA (0.2 mmol/kg); three hours after the administration, animals were subjected to MRI using the same pulse sequence. ROI analysis was performed on T1W-FFE images to calculate aneurysm/muscle ratios (n=3 slices/animal) after correcting for noise. The animals were terminated after the MRI and dissected vessels were and frozen in OCT. The tissue sections were processed for immunohistochemistry using anti-MPO and anti-MAC387 (macrophage-specific) antibodies followed by anti-mouse -AP conjugate with subsequent counterstaining with Nuclear Fast Red.

Results and discussion. To induce local intramural inflammation of the vessel wall in aneurysm model we initially used intramural injection of the LPS solution into the vessel and compared to non-injected controls. The procedure leads to infiltration of MPO-positive neutrophils and MPO-negative macrophages into the site of injection [7]. After the administration of MPO-specific paramagnetic substrate di-5-HT-GdDTPA we observed a visible enhancement of the aneurysms in both LPS-injected aneurysms and saline-injected animals. Post-MPO-specific contrast T1W-FFE MR images demonstrated a difference in aneurysm/muscle normalized signal intensity ratios in LPS group of animals compared to non-injected control animals (1.55 ± 0.05 for LPS versus 1.16 ± 0.10 for control, $P < 0.02$). The evidence of local inflammation induced by control saline injections (a stringent control) due to endothelial damage prompted the development of an alternative procedure involving temporary occlusion and *in situ* incubation of the aneurysms in the presence of LPS vs saline (Fig. 1). Our initial results suggest that the above procedure could potentially lead to more reliable model enabling optimization and imaging of intramural acute inflammation. Our MRI results demonstrated that non-invasive local delivery of test solutions in the close proximity of the aneurysmal wall resulted in a more realistic model that does not require focal vascular damage (Fig 2A), resulting in different enhancement ratios measured in experimental and control animals— 1.35 ± 0.12 (LPS) vs 0.97 ± 0.10 (saline), $P < 0.05$ (Fig. 3) with the concomitant local infiltration of the vessels with MPO-positive cells (Fig. 2).

Conclusion: the development of realistic animal model of cerebrovascular aneurysm enables: 1) testing of inflammation-modulated factors of aneurysmal instability that potentially lead to a rupture in human patients; 2) provide valuable information for further translational studies.

References. 1. Tulamo, R. et al. J Neurointervent Surg 2010 2: 120-130. 2. Querol, M. et al. Org Lett 2005 7:1719-1722. 3. Chen, J. W. et al. Radiology 2006 240: 473-481. 4. Bogdanov, A., Jr. et al. Mol Imaging 2002 1: 16-23. 5. Chen, J. W., et al. Magn Reson Med 2004: 52, 1021-1028. 6. Querol, M., et al. Org. Biomol. Chem. 2005 4: 1887 - 1895. 7. DeLeo, M. J., 3rd et al. Radiology 2009 252: 696-703.

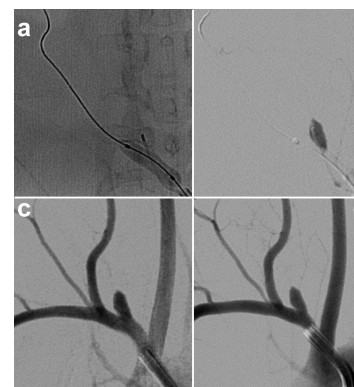


Figure 1. Temporary balloon occlusion of aneurysm with the microcatheter jailed in the aneurysm lumen (A). Selective microcatheter angiography confirms sealing of the aneurysm (B). After injection of LPS solution into the aneurysm, angiography (D) shows no change from baseline angiography (C)

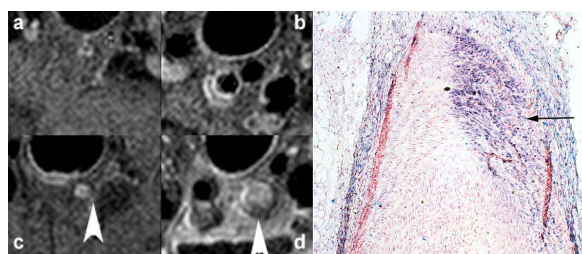


Figure 2. Black blood 3T axial MR images of aneurysm model of a, c- saline and b,d – LPS treated aneurysm (shown with arrows), a, b- pre-contrast; c, d – post IV administration of MPO specific contrast agent.; d- immunohistochemistry of a frozen section showing MPO-positive area (arrow).

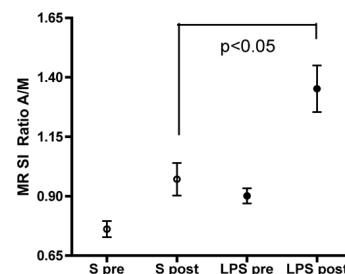


Figure 3. MPO-substrate mediated MR SI aneurysmal enhancement normalized by in-slice signal in the skeletal muscle. Note a statistically significant difference between the ratios measured in saline control (S) and LPS-incubated aneurysms (n=3 MRI slices/animal).