Serial Contrast-enhanced Vessel Wall MRI in a Model of Plaque Neovascularization

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Introduction. Plaque neovascularization, a destabilizing factor in atherosclerosis, serves as a possible source of intraplaque hemorrhage and conduit for inflammatory cells. Measures of plaque enhancement from blood pool MR contrast agents [1-3] have previously been correlated with presence of atherosclerosis, neovessel density and macrophage density. This study introduces an MRI measure of contrast enhancement in the vessel wall that both correlates with these histological measures and monitors plaque development over time.

Methods. Advanced plaques were generated in New Zealand White rabbit abdominal aortas (n = 4) over 20 weeks of cholesterol feeding with endothelial denudation at week 10 and 4 μg/kg injections of vascular endothelial growth factor at weeks 5 and 9 [4]. The animals were imaged at weeks 9, 15 and 20 using a GE 3.0T EXCITE MR system and a 5" custom receive-only coil with a 3-D, axial, high-resolution (374 μm × 374 μm in-plane, 1.6-2.0 mm slice thickness), T1-weighted, fast spoiled gradient-echo sequence. The sequence used SPECIAL fat saturation and a low b value diffusion pulse to attenuate signal from through-plane blood flow. At all time-points, the abdominal aorta was imaged both before and 10 minutes after a gadofosveset injection (0.2 ml/kg, Bayer Schering Pharma, Berlin, Germany).

Vessel wall enhancement area was measured in post-contrast MR slices from week 20 corresponding closest to the centre of each specimen block. Two blinded observers drew ROIs and set a threshold value. The first ROI corresponded to the outer contour of the bright ring that surrounds the dark lumen of the aorta (Fig. 1a+b, solid white arrows) and the second ROI outlined the outmost extent of the vessel wall. Pixels between the two ROIs with intensity values above the threshold were included in the vessel wall enhancement area. The process was repeated for matching MR slices acquired at weeks 9 and 15.

Following sacrifice at week 20, the abdominal aorta was excised, formalin-fixed, and cut into blocks of 5 mm length. Each block was sectioned and stained with H&E, anti-CD31 (Dako Canada, Mississauga, Canada) antibody for endothelial cells, and RAM11 (Dako Canada) for macrophages. For each block, intimal area was measured by tracing the internal elastic lamina and lumen on H&E sections, CD31-positive microvessels within the vessel wall were counted, and RAM11-positive area was segmented based on colour. Average values over each abdominal aorta for both MR and histological measures were compared by finding Pearson's correlation coefficient. Paired t tests were done on the group means of the MR measures from each time point to test for significant differences. A p-value of 0.05 or less was considered significant with all statistical tests performed using SPSS (SPSS, Chicago, USA).

Results. Post-contrast images contained a ring of high signal intensity corresponding to the adluminal surface of the aorta (Fig. 1a-b, solid white arrows). Some of the slices acquired from two animals with neovessel-rich plaques showed surrounding regions that were enhanced but still hypointense to this bright ring (Fig. 1b, green line arrows). CD31 sections corresponding to this enhancement pattern demonstrated extensive neovasculature infiltrating the media and intima (Fig. 1d), while microvessels were confined to the adventitia in the other vessels (Fig. 1c).

MRI-measured vessel wall enhancement area averaged over each aorta demonstrated strong positive associations with microvessel count (R^2 = 0.864, p = 0.136 from one observer), intimal area (R^2 = 0.849, p = 0.151), and macrophage area (R^2 = 0.997, p = 0.003). The group mean of the vessel wall enhancement area showed an increasing trend over all the time points (Fig. 2).

Discussion. Vessel wall enhancement area correlated with intimal area, inflammation, and neovascularization, with all three features contributing to the presence of contrast agent in the vessel wall. The albumin-bound form of gadofosveset would be mostly be found within the plaque neovessels, although inflammation would increase the permeability of these neovessels and facilitate leakage of the bound form. The unbound molecule can enter the plaque from the macrovessel or neovessel endothelia and bind to albumin within the vessel wall, which has been shown to increase with intimal area [5].

Conclusion. MR vessel wall imaging with gadofosveset generates a measure that associates strongly with vessel wall neovascularization, inflammation and plaque size in an animal model of atherosclerosis. This MR measure also tracks changes in plaque complication over time in the same animal.

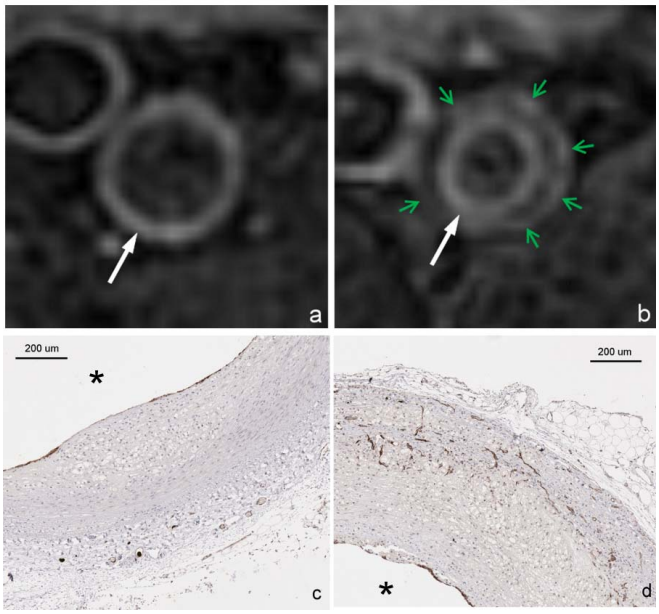

Figure 1 (left). Representative MR images and CD31 (brown stain) sections. (a) Image from an aorta showing no intimal neovascularization. A bright ring (solid white arrow) surrounding the dark lumen indicates the adluminal surface. (b) An aorta showing extensive plaque neovascularization. Surrounding the adluminal bright ring (solid white arrow) are additional enhancing regions (green line arrows). (c) An artery that developed moderately sized plaques with vasa vasorum confined to the adventitia. (d) An artery that formed large plaques with neovessels infiltrating the inner layers. (*) denotes lumen.

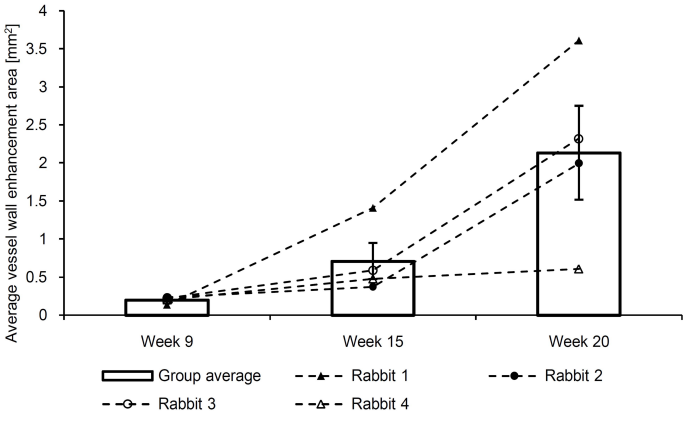

Figure 2 (above right). Changes in average vessel wall enhancement area as measured by Observer 1 over time, with data shown at week 9 (before endothelial denudation), week 15 (5 weeks after endothelial denudation), and week 20 (time of sacrifice). Averages for each animal are plotted, along with the group average and its standard error for each time point. The paired t test results indicated a significant difference in the group average between weeks 15 and 20 for Observer 1 (p = 0.05), but not for Observer 2 (p = 0.075), and not between weeks 9 and 15 for either observer.

References. [1] Cornily J, Hyafil F, Calcagno C, et al. J Magn Reson Imaging. 2008;27(6):1406-1411. [2] Lobbes MBI, Miserus RJHM, Heeneman S, et al. Radiology. 2009;250(3):682-691. [3] Sirol M, Moreno P, Purushothaman KR, et al. Circ Cardiovasc Imaging. 2009. [4] Chiu SE, Moody AR, Zhan JQ, Leung G. Proc of the 17th ISMRM. 2009. [5] Zhang Y, Cliff WJ, Schoefl GI, Higgins G. Am J Pathol. 1993;143(2):496–506.