Characterization of an *in vivo* model of atherosclerosis using histological and MRI techniques

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Introduction Intraplaque hemorrhage and plaque neovascularization are recognized as contributors to atherosclerotic plaque vulnerability [1], but current animal models do not consistently or spontaneously produce these types of lesions. As a first step towards building upon the commonly used hypercholesterolemic rabbit model [2], a low dose of recombinant human vascular endothelial growth factor (VEGF) was administered to a group of rabbits. These injections have been shown to increase both intramural endothelial cell and macrophage density in the abdominal aortas of hypercholesterolemic rabbits [3]. MRI was performed on the rabbits in an attempt to detect these changes non-invasively.

Methods Two different groups of New Zealand white rabbits (n=4 each) were fed a 6% peanut oil and 0.25% cholesterol diet for 20 weeks. One group was given 2 µg/kg intramuscular injections of VEGF (R&D Systems) at weeks 5 and 10, while the other served as a control. Both groups were imaged at week 20 using a GE 3.0T EXCITE MR system and a 5" custom receive-only coil, and were sacrificed within the week. On two separate days, the abdominal aorta in each rabbit was imaged with a 3-D, axial, high-resolution (234 µm × 234 µm in-plane), T1-weighted, fast spoiled gradient-recalled echo sequence both before and 3 minutes after contrast injection (0.2 ml/kg). The sequence has a low *b* value diffusion pulse to attenuate the signal from through-plane blood flow [4]. Magnevist and Vasovist (both Bayer Schering Pharma) were administered on separate days. Following sacrifice, the abdominal aorta from the renal arteries to the iliac bifurcation was excised, fixed in 10% neutral buffered formalin, and cut into blocks of 5 mm length.

From each tissue block, 3 contiguous sections of 5 µm thickness were cut and stained with H&E, anti-CD31 (Dako Canada) for endothelial cells, and RAM11 (Dako Canada) for macrophages. For each set of slides, total macrophage-positive area (Figure 1) was measured and CD31-positive blood vessels (Figure 2) in the aorta wall with a diameter of less than 50 µm were counted. For each rabbit, the values taken from each set of slides along the aorta were averaged and normalized to the number of slices measured.

To analyze vessel wall enhancement, the pre-contrast images were subtracted from the post-contrast images, with the result then normalized to the mean spinal cord intensity in each pre-contrast image. The average intensity in the difference image for several evenly-spaced slices throughout each acquired volume was measured over a manually-segmented region of interest encompassing the vessel wall, based on the corresponding post-contrast image.

Results The overall averages, standard errors, and Student's *t* test probabilities were found within each group of rabbits (Table 1). As expected, there was a significant increase in the vessel count for the VEGF group as compared with the control group. However, the differences in macrophage-positive area, Magnevist enhancement, and Vasovist enhancement did not reach significance.

Discussion and Conclusions As Vasovist is an intravascular contrast agent, Vasovist wall enhancement was expected to correlate with vessel count. While there was a corresponding increase in Vasovist enhancement in the VEGF group, this increase was non-significant, perhaps owing to the poor resolution relative to aorta wall thickness and the low sampling rate along the aorta. Macrophage area was not significantly different between the groups, contrary to previous results. The relationship between Magnevist enhancement and histological parameters remains unclear, as Magnevist enhancement likely depends on vessel wall permeability in addition to microvessel density. More refined imaging and analysis techniques will be required to further elucidate the relationships between the imaging and histological parameters.

References

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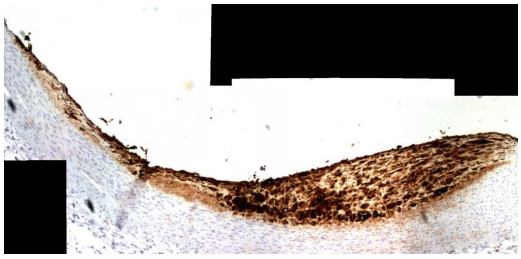

Figure 1 (left). Macrophage-positive area (dark brown) within an aortic plaque.

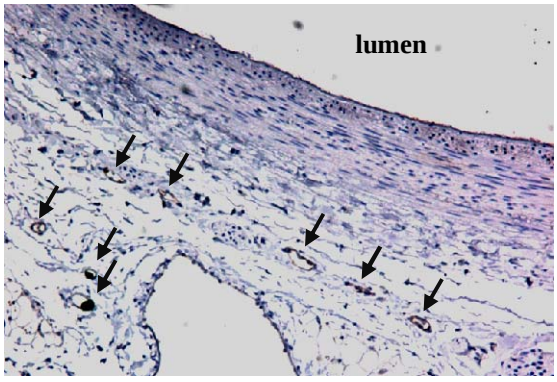

Figure 2 (right). Small vessels (arrows) stained for CD31 in the vessel wall.

	Control Group		VEGF Group		*p* value
	Average	Standard Error	Average	Standard Error	
CD31+ vessels	7.80	0.73	12.32	1.09	0.017*
Vasovist enhancement	1.16	0.07	1.32	0.17	0.45
Macrophage+ area [mm^2]	0.0193	0.0065	0.0126	0.0091	0.57
Magnevist enhancement	0.77	0.07	0.62	0.06	0.17

Table 1. Control and VEGF group averages for histological and MR parameters. * denotes a significant difference between groups