In-vivo Serial Assessment of Aortic Aneurysm Formation in Mouse

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Introduction

Abdominal aortic aneurysms can be consistently produced in hyperlipidimic mice by continuous infusion of Angiotensin II (Ang-II) [1,2,3]. One characteristic in aneurysm development is the degradation of the extracellular matrix through one or more matrix metalloproteinases (MMPs)[2]. The morphology of these aneurysms has been studied *ex-vivo* using histological staining, while their development has been examined *in-vivo* using ultrasound [3]. However, these techniques are lacking in that neither provides the ability to track the aneurysm's progression and morphological changes *in-vivo* at high spatial resolution. MRI allows the tracking of aneurysm development *in-vivo* with sufficient spatial resolution to characterize morphological changes and make accurate measurements of aneurysm size. The addition of bright-blood contrast allows the visualization of medial breaks, an additional index of aneurysm severity. For this study we used MRI to characterize aneurysm development in a murine model. In addition we examined the effect of the broad based MMP inhibitor, doxycycline, on the onset and progression of aneurysms over a four week period. This is, to the authors' knowledge, the first use of MRI to study *in-vivo* abdominal aortic aneurysm progression in the mouse.

Methods

Osmotic pumps were implanted subcutaneously in ApoE -/- mice for continuous infusion of Ang-II (1000 ng/kg/min) over a four week period. Animals were given 30 mg/kg/day doxycycline or vehicle (n=15, both groups) via drinking water. Weekly scans were performed using a 9.4T small animal vertical-bore magnet (Bruker Biospec, Billerica, MA). Coronal scout images were acquired to determine location of the abdominal aorta and renal branches (TR=15 ms, TE=2.7 ms, 256X256 matrix, 0.12X0.12X1 mm voxels, NEX=16). Bright-blood gradient echo scans were performed through the transverse plane of the abdominal aorta, covering 10 mm proximal to the right renal branch (TR=15 ms, TE=2.7 ms, 256X256 matrix, 0.12X0.12X1 mm voxels, 10 slices, NEX=8). Necropsy was performed on animals which died during the course of the study and surviving animals were sacrificed at the end of study for further tissue analysis.

Images were examined to determine the presence of aneurysms. Size comparisons were performed off-line (Analyze (Biomedical Imaging Resource, Mayo Clinic, Rochester MN)) by identifying the area of the largest section of aneurysm at onset and following that section for subsequent scans. Aneurysms were classified as having a medial break based on the presence of bright blood outside of the aortic lumen and within the aneurysm.

Results and Discussion

Images of transverse cross sections through the abdominal aorta are shown in Figure 1. At baseline (Figure 1A) there is no aneurysm present and the lumen appears concentric with a diameter of 1.42 mm. Figure 1B and C demonstrate the two distinct features of aortic aneurysms described by Daugherty et al. [1]: remodeling of the adventitia which compresses the aortic lumen (B) and a break in the medial wall (C) characterized by bright-blood signal within the aneurysm.

Comparison of the vehicle and doxycycline groups showed a larger percentage of aneurysms in the vehicle group for each weekly scan (Figure 2 A). The vehicle group also produced aneurysms at a faster rate than the doxycycline group and comparing the number of aneurysms detected by MRI and necropsy for both groups showed a more rapid time-to-aneurysm in the vehicle group (log-rank test, $P<.05$). The size of the aneurysms in the vehicle group was significantly larger than in the doxycycline group ($4.02\pm.37mm^2$ vs. $2.56\pm.32mm^2$, $P<.01$) after four weeks of Ang-II infusion. Temporal changes in aneurysm size were similar in both groups, (correlation of mean corrected curves r=.93) however the vehicle group produced consistently larger aneurysms (Figure 2B). In addition to onset of aneurysm and aneurysm size, MRI also allowed the detection of bright blood within the aneurysm, indicating a break in the medial wall of the aorta. Over the course of the study, the number of aneurysms that showed a medial break was greater in the vehicle group (100%) vs. doxycycline (42.86%).

The use of MRI for visualizing the onset and progression of abdominal aortic aneurysms in the murine model has been demonstrated for the first time. Differences in aneurysm development and morphology due to pharmacological treatment could be assessed. These results demonstrate MRI's potential as a tool for studying the pre-clinical aneurysm model of atherosclerotic inflammation *in-vivo*.

References

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3. Barisione C et al., J Vasc Surgery, 44:372-376, 2006

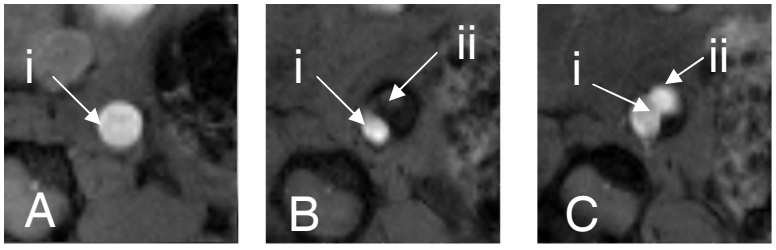

Figure 1: MR images of transverse cross-sections of the abdominal aorta. A) At baseline, the aorta (i) appears concentric and no aneurysm is present. B) The aorta appears compressed due to the presence of an aneurysm. The aneurysm (ii) appears dark compared to the bright blood within the aorta, indicating remodeling of the adventitia with no medial break. C) Bright signal indicates there is a break in the medial wall of the aorta (i) allowing blood to flow into the aneurysm (ii).

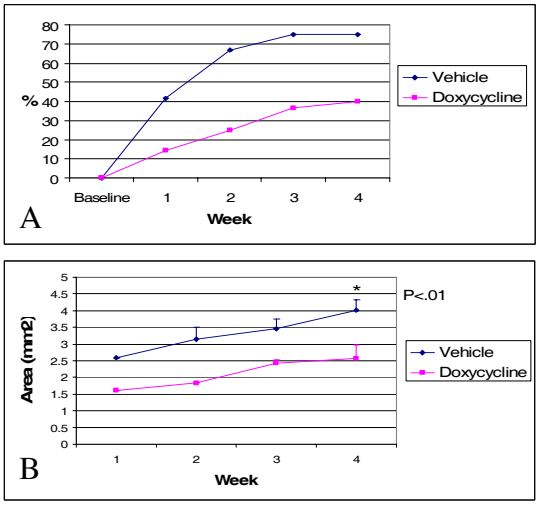

Figure 2: A) Percent of aneurysms detected weekly via MRI in the vehicle and doxycycline groups over four weeks. B) Average cross-sectional area of aneurysms measured by MRI.