Combining contrast enhanced in vivo and ex vivo MRI for characterizing vulnerable plaques in a rabbit model of atherothrombosis

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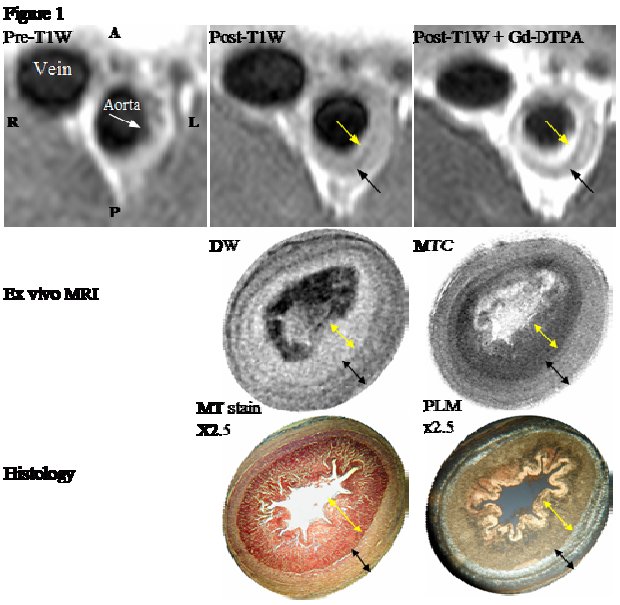
Introduction

Atherosclerosis is a chronic disease of the arteries characterized by the accumulation of lipids, inflammatory cells, and molecules in the sub-intimal layer. Some atherosclerotic lesions progress for years without symptoms, while other lesions, termed "vulnerable plaques," can spontaneously rupture with subsequent thrombosis. Plaque rupture is the cause of most acute coronary syndromes and strokes wgich are the leading causes of death in the US. It is therefore important to identify vulnerable plaques prior to rupture. However, this cannot be done in controlled studies with humans. Here we study plaque rupture in a rabbit model, the Constantinides New Zealand White (1), which permits triggering of plaques with chemical injections at a precise time. We have combined contrast enhanced (Gd-DTPA) *in vivo* (3T) and *ex vivo* (11.7T) MRI together with histology to identify thrombus and other characteristics associated with vulnerable plaques.

Materials and Methods

NZW rabbits were fed a 1% high cholesterol diet, 2 weeks prior to and 6 weeks after balloon injury, followed by 4 weeks of normal diet. Plaque rupture was induced by injecting Russell's viper venom (0.15 mg/kg IP) followed by histamine 30 min later (0.02 mg/kg IV). This procedure was repeated 24hr later. *In vivo* MR experiments were performed on a 3T Philips Intera Scanner before and 24hrs after the final injections using a synergy knee coil with 6 elements. Axial (4mm) T1BB (TR=2 cardiac cycles, TE=10ms, TSE=15) images were acquired using a black-blood, double inversion recovery, TSE sequence with cardiac gating. NEX=2, in-plane resolution=122x122µm was used for all *in vivo* images. Contrast-enhanced T1BB images were repeated 10min after injection of Gd-DTPA (0.1mmol/kg IV). *Ex vivo* MR experiments were performed on an 11.7T Bruker Avance Scanner using a 10mm birdcage coil. For registration between *in vivo* and *ex vivo* images, the branch of the aorta to the left kidney was used as an anatomical marker. Spoiled GRE images with and without magnetization transfer were acquired with TR=330ms, TE=4ms, flip angle=30°, NEX=128, slice thickness=0.5mm and in-plane resolution=24x24µm. The MT pulse was applied 10000 Hz off-resonance with a duration=12ms and power=10µT. The magnetization transfer contrast (MTC) was revealed by subtracting the image without MT from the one with MT. The parameters for the DW images were TR=1s, TE=25ms, NEX=32, Δ=12.6ms, δ=5ms, in-plane resolution=0.5x0.5µm and slice thickness=1mm. The ADC was calculated from different b-values=0, 196, 442, 637, 867, 1770 and 2409 s/mm^2. Histology was performed on 10µm frozen sections.



Results and Discussion

Figure 1 shows *in vivo* T1W images (before trigger without Gd-DTPA, after trigger without and with GTPA), *ex vivo* DW and MTC images, and histology. All the slices were taken at the same level of the aorta. The *in vivo* pre-T1W image shows luminal narrowing of the abdominal aorta with an eccentric atherosclerotic plaque (white arrow). The post-T1W image shows a new feature in the lumen that is slightly hyperintense (yellow arrow) overlaying a region of lower intensity (black arrow). The increased occlusion is due to a thrombus that formed after plaque rupture and remained attached to the plaque. However, the thrombus is not clearly distinguished from the vessel wall except from a dark region representing lipid in the plaque, as shown by histology (PLM). However, after addition of Gd-DTPA the thrombus is highlighted and easily distinguished (yellow arrow). The *ex vivo* images provide a higher resolution and confirm the presence of a thrombus overlaying the plaque. In fact, the thrombus appeared to be more occlusive probably because of recruitment of additional red blood cells after the rabbit was sacrifice. The DW image (b-value=442s/mm^2) shows the thrombus as a bright area (yellow arrow) adjacent to the lumen, while the vessel wall with the plaque has a darker (black arrow) appearance. Thus, these components have different ADC values (table 1), which can be used as the basis of their differentiation along with standard T2 and T1 relaxation times. An additional contrast method to differentiate thrombus from the underlying plaque is magnetization transfer. The MTC image shows a reversal of the DW image appearance, as expected, and the thrombus is now darker. Histology [masson's trichrome (MT x2.5)] confirmed our image assignment of an organized thrombus. Polarized light microscopy (PLM x2.5) of an unstained, section shows that the intima of the plaque (black arrows) is filled with lipid-rich macrophages (bright band) that are in direct contact with the thrombus after the plaque ruptured. These are the hallmark features of plaque rupture as also seen in post-mortem human plaques using histology.

We have shown that luminal thrombi, formed after triggering plaque rupture, as well as the lipid core can be identified *in vivo* on post-T1W images and on high resolution *ex vivo* DW and MTC images. The use of Gd-DTPA for *in vivo* experiments highlights the thrombus and facilitates delineation of thrombus from the vessel wall. Future application of *ex vivo* DW and MTC protocols to *in vivo* experiments will provide further insights to plaque vulnerability.

Table 1	ADC mm^2/s	T2 ms	T1 ms
Platelet/Fibrin rich thrombus	0.5±0.02	17±1	1565±30
Lipids	0.3±0.01	16±1	1614±29

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References: 1.Constantindes, P.C. 1961. Rabbit Arterial Thrombosis Production by Systemic Procedures. *Archives of Pathology* 72:197-208.