In vivo contrast-enhanced (Gd-DTPA) and ex vivo magnetization transfer and diffusion weighted MRI detect changes in thrombus composition during propagation from sites of disrupted atherosclerotic plaques.

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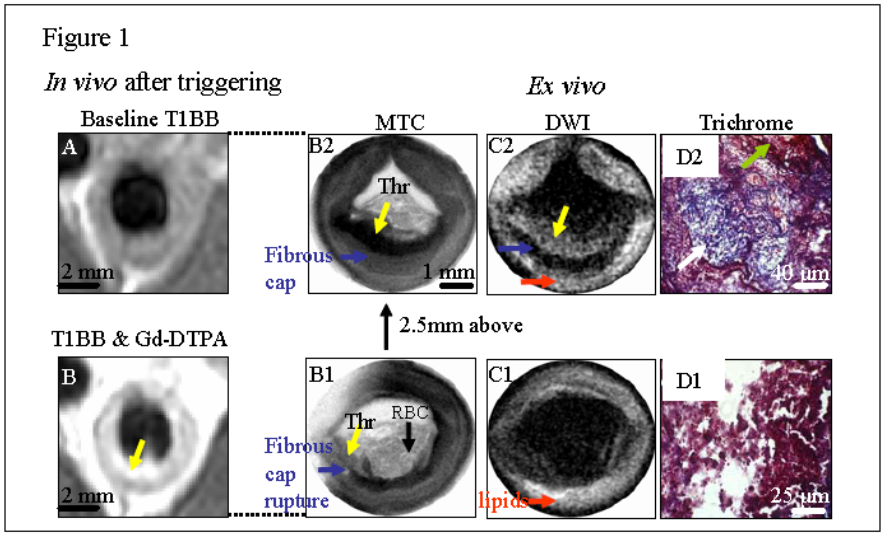
Introduction: Acute coronary syndromes (ACS) such as unstable angina pectoris, myocardial infarction and sudden death are the major cause of mortality in industrialized nations (1). Extensive histological studies have demonstrated that most of ACS are triggered by rupture/erosion of vulnerable atherosclerotic plaques which result in luminal thrombosis (2). However, little is known about the factors that determine the composition of the thrombus, how far it will propagate in along the lumen, and whether it will be occlusive. To address these questions, which are not amenable to controlled study in humans, we studied plaque disruption in a rabbit model in which thrombosis is experimentally controlled by a combination of *in vivo* (3T) and *ex vivo* (11.7T) MRI (with and without Gd-DTPA) coupled to histology.

Materials and Methods: New Zealand White rabbits were fed a 1% cholesterol diet 2 weeks prior to and 6 weeks after balloon injury, followed by 4 weeks of a normal diet. Plaque disruption 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 24h after the first injections. *In vivo* MR experiments were performed on a 3T Philips Intera Scanner using a synergy knee coil with 6 elements before (pre) and 48h after (post) pharmacological triggering. T1BB (TR=2 cardiac cycles, TE=10ms, TSE=15). MR images were acquired using a black-blood, double inversion recovery, TSE sequence with cardiac gating. Axial images were acquired with 4mm slices, NEX=2, in-plane resolution=234x234µm Contrast-enhanced T1BB images were repeated 10min after injection of Gd-DTPA (0.1mmol/kg IV). After the *in vivo* MRI, the extracted aorta was fixed in 10% formalin. *Ex vivo* MR experiments were performed at 11.7 T Bruker Avance using a 10mm birdcage coil. For registration between *in vivo* and *ex vivo* images, the renal branches and the iliac bifurcation were used as anatomical markers. Spoiled GRE images with and without magnetization transfer were acquired with: TR=330ms, TE=4ms, flip angle=30°, NEX=192, slice thickness=0.5 mm, MTX=256x256 and in-plane resolution=24x24µm. The MT pulse was applied 10000 Hz off-resonance with a duration=12ms and power=20µT. The % magnetization transfer rate (MTR) was calculated as follows: (Image $_{\text{with MT}}$-Image $_{\text{without MT}}$/Image $_{\text{with MT}}$)*100. *Ex vivo* DW images were acquired with: 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 cross-sections stained with Masson's trichrome.



Results and Discussion:

Examination of disrupted aortic plaques (n=16) revealed that thrombi propagated both parallel and anti-parallel to blood flow. *In vivo*, *ex vivo* MR images and histology of a ruptured atherosclerotic plaque with thrombus propagation anti-parallel to the direction of blood flow are shown in Figure 1. The baseline T1BB (Fig. 1A) image shows concentric vessel wall thickening before and after infusion of Gd-DTPA (Fig. 1B) the thrombus is enhanced (yellow arrow). For a more detailed study, two thinner, *ex vivo* slices corresponding to the *in vivo* slice were acquired with MT (Fig. B1-B2) and DW (C1-C2) imaging. The MTC image identified the site of fibrous cap rupture as a discontinuity of the fibrous cap (dark band) (Fig. B1; blue arrow) and the attachment of a thrombus (yellow arrow). The lumen appears more occluded compared to the *in vivo* image because of post-mortem red blood cell aggregation (RBC; black arrow, gray intensity). The corresponding DWI (Fig. C1) detected the lipid core underlying the fibrous cap. Histology (Fig. D1) revealed that the thrombus attached to the rupture site is composed of aggregated platelets with little fibrin. The MTC image acquired from the slice located 2.5 mm above (Fig. B2, yellow arrow) shows that the thrombus has propagated into the lumen from the site of plaque rupture in a direction anti-parallel to blood flow and spread circumferentially over a relatively thick fibrous cap (Fig. B2; blue arrow). The thrombus and the lipid-core are highlighted on the DW image while the signal from the collagen–rich fibrous cap is attenuated (Fig. C2). The corresponding histological section shows that at the leading edge of propagation the thrombus was enriched in fibrin (white arrow) and red-blood cells (green arrow). The changes in thrombus composition are reflected on the calculated % MTR and D coefficients which were: 9.5±2 % and 0.52±0.05 mm^2/s at the site of rupture and 43±1 and 0.4±0.05 at the propagated site.

In Conclusion, changes in thrombus composition during propagation were demonstrated by the combination of Gd-DTPA contrast-enhanced *in vivo* and MT and DW images ex *vivo* coupled to histology. At the site of rupture, the thrombus was rich in platelets. However, at distal sites the thrombus was enriched in fibrin and circulating red-blood cells. Whether thrombi related to disrupted plaques become occlusive is crucial to the onset of clinical events. *In vivo* identification of plaque constituents and hemodynamic factors that might regulate this process is important and may permit a site-specific assessment of risk for acute cardiovascular events which could confer more specificity in treatment options.

References: **1**.Braunwald, E. *N Engl J Med*; 1997;337(19):1360-9. **2.** Van Der Wal *et al. Circulation* 1994;89:36-44. **3**. Constantinides, P. *et al. Arch. Pathol.* 1961;72:197-208.