New insights into ruptured plaques: enhanced detection of neovasculature and fibrous tissue by MRI.

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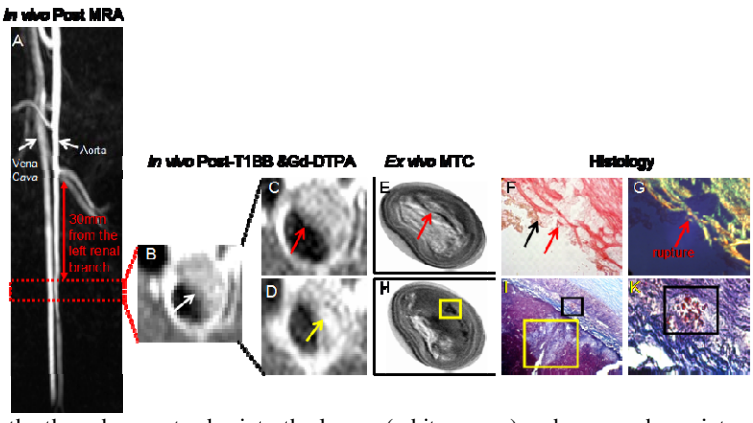
Introduction: Atherosclerosis is a major contributor to acute cardiovascular events, which remain the leading cause of death in industrialized nations (1). Stable atherosclerotic plaques that partially obstruct the lumen may remain asymptomatic for years. In contrast, vulnerable lesions can spontaneously rupture resulting in the formation of mural thrombi which can cause sudden death (2). These complex pathological processes are impossible to study in a controlled manner in humans. Thus, we studied plaque rupture in the Constantinides New Zealand White (3) rabbit model, which permits pharmacological triggering of plaque rupture at a precise time point. Previous studies suggested that Gd-DTPA may help to identify plaque neovasculature which is believed to be associated with infiltration of inflammatory cells and plaque destabilization (4) as well as regions rich in fibrous tissue (5). We combined contrast enhanced (Gd-DTPA) *in vivo* (3T) and *ex vivo* (11.7T) MRI together with histology to identify morphological and structural features 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 a 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 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 24hrs after (post) the second pharmacological triggering. Phase contrast MRA was used to identify the abdominal aorta and its renal branches. Typical experimental parameters were: TR=20ms, TE=3.5, flip angle=15°, NEX=2, slices=25, slice thickness=1mm, flow velocity=75 cm/s, MTX=256x244 (in-plane resolution=586x586µm) and scan time=3 minutes. 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 and 2mm slices with NEX= 2 and in-plane resolution=117x177µm. Contrast-enhanced T1BB images were repeated 10min after injection of Gd-DTPA (0.1mmol/kg IV). After the MRI, the extracted aorta was preserved in 10% formalin solution and stored at 23°C. *Ex vivo* MR experiments were performed on an 8.9cm bore 11.7 T (500MHz for ^{1}H) Bruker Avance using a 10mm birdcage coil. For registration between *in vivo* and *ex vivo* images, the left renal branch of the abdominal aorta 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=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 contrast (MTC) was calculated by subtracting the image without MT from the one with MT. Histology was performed on 10µm cryo-sections which were stained with Masson's trichrome and Picrosirius red, for identifying cellular components and types of collagen fibers, respectively.



Results and Discussion: *In vivo* and *ex vivo* MR images and histology of a rupture rabbit aortic atherosclerotic plaque are shown in Figure 1. The MRA (Figure 1A) identifies the vena cava and the descending abdominal aorta with its renal branches. The red rectangle indicates the location of axial slices, acquired after pharmacological triggering (Figures 1B-D). Figures 1B-D are T1BB images after Gd-DTPA infusion. Figure 1B (4mm) shows that the thrombus protrudes into the lumen (white arrow) and appears hyperintense. Thinner slices (2mm) with higher resolution slices taken at the level of Figure 1B reveal additional structural details about the plaque (Figures 1C-D): a prominent surface bulge (Figure 1C; red arrow) and a hyperintense region within the plaque (Figure 1D, yellow arrow). The higher resolution *ex vivo* MTC MR images (Figures 1E-H) show key features of ruptured plaque, a discontinued fibrous cap (Figure 1E, red arrow), and fibrosis within the thrombus (Figure 1H).

The corresponding histology (Figures 1F and 1G) verified the location where the collagen fibers ruptured (Figure 1F-red and Figure 1G-yellow; red arrows). Figure 1F also demonstrates the beginning of a thrombus attached at the site of plaque rupture (black arrow). This location may correspond to the surface bulge identified in the *in vivo* MR image (Figure 1C). Histology for the *ex vivo* image shown in figure 1H revealed areas of fibrosis within the thrombus (yellow box), which is a characteristic of well organized/old thrombi (Figure 1I). This is consistent with our prediction that hypointense regions in the MT image reflect organized fibrous protein (Figure 1K). Higher magnification histology revealed the presence of red blood cell filled neovessels within the plaque (Figure 1I). The combination of neovasculature and fibrosis could explain the hyperintense region seen after the injection of Gd-DTPA in Figure 1D. Neovessels could help deliver Gd-DTPA into the plaque which is then restricted within regions rich in fibrous tissue.

In Conclusion, we have shown that the combination of Gd-DTPA contrast-enhanced *in vivo* and magnetization transfer *ex vivo* MR images coupled to histology provides a valuable strategy to reveal morphological characteristics of atherosclerotic plaques. Gd-DTPA uptake was increased in plaques with neovasculature as well as regions rich in fibrous tissue. *Ex vivo* use of MT detected fibrous cap rupture and fibrosis within organized thrombus.

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