# MRI-detected Intraplaque Hemorrhage in an Animal Model

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**Introduction.** Intraplaque hemorrhage (IPH) is recognized as a plaque destabilizing factor, due to the myriad proatherosclerotic effects of red blood cells (RBCs) [1]. A high resolution, fat- and flow-suppressed, T1-weighted MRI sequence (MRIPH) has been shown to detect IPH due to the T1-shortening effects of methemoglobin. Presence of MRIPH positive signal predicts future ischemic events in both symptomatic [2] and asymptomatic [3] carotid patients. An animal model of IPH has the potential to study the natural history of IPH at selected time points and MRI as a noninvasive tool can track MRIPH signal evolution in a serial fashion. However, the suitability of animal models for the study of *in vivo* MRIPH signal to our knowledge has not been previously explored. This study presents the first evidence of MRI-detected IPH in an animal model of simulated IPH.

**Methods.** Advanced plaques were generated in New Zealand White rabbit abdominal aortas ($n$ = 3) using a 2% cholesterol and 6% peanut oil diet and endothelial denudation with an arterial embolectomy balloon catheter. Initiation of the diet was defined as week 0 and denudation was performed at week 2. The 2% cholesterol diet was continued until week 5, followed by a 0.25% cholesterol and 6% peanut oil diet from weeks 6 to 16 and then normal chow for the remaining time. The animals were imaged at week 15 using a GE 3.0T EXCITE MR system and a 5" receive-only coil with a 3-D, axial, T1-weighted, fast spoiled gradient-echo sequence (375 μm × 375 μm in-plane, 1.6 mm slice thickness, TE/TR/θ = 3.1 ms/10.7 ms/15˚) and a 3-D, axial, T1-weighted spin echo sequence (375 μm × 375 μm in-plane, 2 mm slice thickness, 4 mm spacing, TE/TR = 25 ms/567 ms). SPECIAL fat saturation was used in both sequences. The abdominal aorta was imaged with both sequences before and immediately after a gadolinium contrast agent injection (0.2 ml/kg, Magnevist, Bayer Schering Pharma, Berlin, Germany).

Rabbits underwent catheterization with a needle injection catheter (modified Bullfrog, Mercator MedSystems, San Leandro, USA) at week 17 or 19. Approximately 25 μl of washed autologous RBCs suspended in 25 μl of saline and iodinated contrast agent (Isovue, Bracco Diagnostics, Inc., Mississauga, Canada) mixture were injected into the vessel wall under fluoroscopic guidance via femoral artery access. Injection sites were chosen based on areas of vessel wall enhancement in the post-contrast week 15 MR images since these areas correspond with plaque neovascularization and inflammation [4]. Upon inflation of the actuating balloon of the catheter, the 35G, 0.4 mm length microneedle was exposed and pushed in the opposing vessel wall, allowing for intramural delivery. Specimens were collected for histology from each of the time points (one rabbit each), while the remaining week 19 rabbit was imaged with MRI a week later as described before being sacrificed. The abdominal aorta was excised, formalin-fixed, and cut into blocks of 5 mm length. Each block was sectioned and stained with H&E.

**Results.** Post-contrast images from week 15 showed MRI plaque enhancement indicative of plaque inflammation and neovascularization in all three aortas, suggesting advanced atheroma had developed prior to RBC injection (Fig. 1, top right panel). H&E sections confirmed the successful delivery of intact RBCs into the vessel wall of each aorta (Fig. 2a). The H&E sections revealed the presence of neovessels in the media and intima of all vessels, as well as regions of foam cells and cholesterol clefts, fibrous tissue, and calcification. The MR images from the one rabbit imaged one week post-injection (Fig. 1, bottom row) showed a region of high signal intensity within the vessel wall in several contiguous slices (in the case of the gradient echo images). This region corresponded to the injection site as chosen under fluoroscopic guidance as well as the H&E section showing hemorrhage products in the vessel wall (Fig. 2b).

**Discussion.** The needle injection catheter successfully delivered RBCs to advanced rabbit aorta plaques featuring plaque neovascularization, extensive inflammation, lipid cores, and fibrous caps. Histology obtained from one week after injection showed that RBCs and their products remained in the vessel wall. Contrast-enhanced MRI at week 15 indicated that large, neovessel-rich plaques formed in the abdominal aorta of all 3 subjects, providing a suitable environment for simulating IPH via injection of packed RBCs. T1-weighted MR images obtained one week after IPH induction showed a hyperintense region at the site of injection, indicating that hemoglobin from the RBCs remained in the vessel wall and was oxidized to methemoglobin, a T1-shortening species, within 7 days.

**Conclusion.** Within 7 days of intramural injection of washed, intact RBCs into advanced abdominal aorta plaques, the RBCs remain within the vessel wall. T1-weighted MRI at this time point detects bright signal in the vessel wall at the injection site that is presumably due to the formation of methemoglobin. The similarity of this T1-weighted MR signal to that in patients suggests that the described model of simulated IPH is suitable for imaging studies of IPH. To our knowledge, this is the first demonstration of IPH in an animal model imaged with MRI.

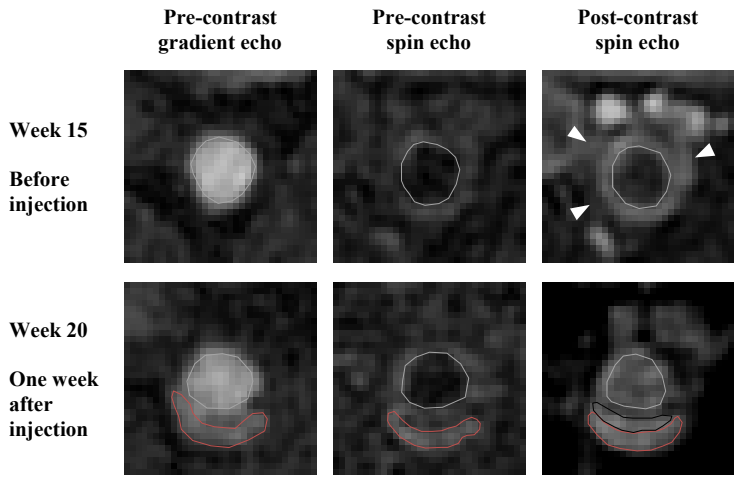


**Figure 1 (above).** Matching MR images at the location of RBC delivery acquired 4 weeks before (top row) and 7 days after (bottom row) injection. Lumen (grey outline) appears bright in gradient echo images due to inflow while flowing blood is suppressed in pre-contrast spin echo images. Top row: pre-contrast images before injection show vessel wall iso- or hypointense with muscle, indicating absence of pooled methemoglobin. Post-contrast spin echo before injection shows areas of vessel wall contrast enhancement (arrowheads) that indicate plaque neovascularization and inflammation. Bottom row: both gradient echo and spin echo images reveal vessel wall hyperintensity (red outline) characteristic of IPH. Post-contrast image contains additional regions of enhancement (black outline) possibly indicative of increased inflammation and/or neovascularization at this later time point. Each panel is 7.5 mm × 7.5 mm.

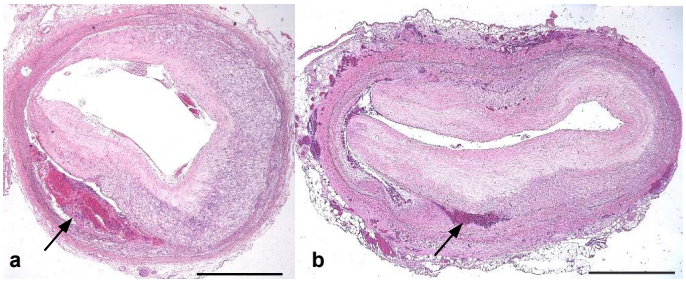


**Figure 2 (above).** H&E sections from the site of injection immediately (a) and 7 days (b) after injection. (a) A pool of intact red blood cells (arrow) remains in the medial layer of the vessel wall, deep to the fibrous cap of the plaque. (b) Evidence of hemorrhage (arrow) is still present within the vessel wall one week after injection. Bars indicate 1 mm.

**References.** [1] Virmani R, Kolodgie FD, Burke AP, et al. Arterioscler Thromb Vasc Biol. 2005;25:2054-61. [2] Altaf N, Daniels L, Morgan PS, et al. J Vasc Surg. 2008;47:337-42. [3] Singh N, Moody AR, Gladstone DJ, et al. Radiology. 2009;252:502-8. [4] Chiu SE, Moody AR, Zhan JQ, Leung G. Proc of the 18th ISMRM. 2010.