Molecular MRI and fluorescence imaging of atherosclerosis using annexin A5-functionalized bimodal nanoparticles

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Introduction

Apoptosis and macrophage burden are believed to correlate with plaque vulnerability [1-2]. Viable macrophages as well as apoptotic cells are both known to expose phosphatidylserine (PS) at the cell surface, while this phospholipid in most non-pathological cells is restricted to the inner leaflet of the cell membrane. Annexin A5 is a 36 kDa protein that binds with high affinity to PS. Therefore, in the present study we investigated the use of an annexin A5-functionalized contrast agent for non-invasive magnetic resonance imaging (MRI) of PS-exposing cells in atherosclerotic lesions. To that aim we developed and applied a bimodal micellar contrast agent, composed of PEGylated, paramagnetic and fluorescent lipids.

Materials and Methods

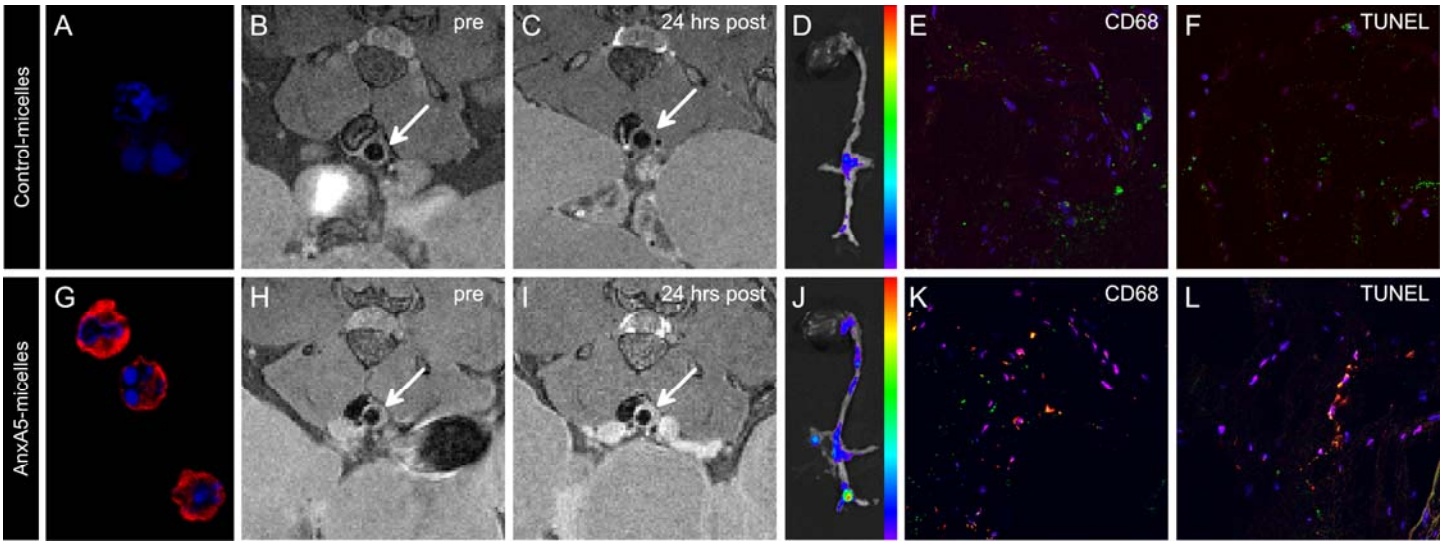
Bimodal micelles, with a hydrodynamic diameter of ~15 nm, were composed of Gd-DTPA-BSA (50%), PEG2000-DSPE (39%), maleimide-PEG2000-DSPE (10 %), and near-infrared (NIR) Cy5.5-PEG-DSPE (1%). The protein annexin A5 (anxA5) was covalently conjugated to obtain specificity for PS. ***In vitro:*** Apoptotic Jurkat cells were incubated with untargeted control-micelles or anxA5-micelles (1mM total lipid, 30 min.), fixed, stained for nuclei (DAPI) and studied with confocal laser scanning microscopy (CLSM). ***In vivo:*** 10 male apoE$^{-/-}$ mice (60-68 weeks of age, high cholesterol diet) were used for *in vivo* MRI experiments and additional *ex vivo* fluorescence imaging or microscopy. Mice received 2.5 µmol control-micelles (n=4) or anxA5-micelles (n=6). T_1-weighted MR images of the abdominal aorta were acquired pre-contrast and 24 hrs post-contrast at 9.4T, using a fat-suppressed black blood spin echo sequence (TR/TE= 800/8.6 ms, NEX = 16, 101.6 x 101.6 µm^2, 0.5 mm slice thickness). Following MRI, aortas were dissected and near-infrared fluorescence reflectance images of whole aortas were acquired with identical exposure times for both groups and a control aorta. Next, aortas were cryo-sectioned, stained for apoptotic cells (TUNEL) or macrophages (CD68) and studied with CLSM.

Results

Target-specificity of the anxA5-micelles was confirmed with *in vitro* binding assays to apoptotic Jurkat cells (compare A and G). *In vivo* T_1-weighted MRI of the abdominal aorta in apoE$^{-/-}$ mice at 24 hours post-injection of the anxA5-micelles revealed a modest increase of the mean signal intensity in the aortic wall compared to pre-contrast levels (10.7 ± 1.7%; compare H and I). The signal intensity was less increased at 24 hours after injection of control-micelles (6.7 ± 3.4%; compare B and C). *Ex vivo* near-infrared fluorescence imaging of excised whole aortas demonstrated most pronounced uptake of the annexin A5-micelles (compare D and J), and predominantly in areas that were rich of atherosclerotic plaque [3], such as the aortic bifurcation into the iliac arteries. Furthermore, confocal laser scanning microscopy (CLSM) revealed that the targeted agent was associated with macrophages (K) and apoptotic cells (L), whereas the non-specific control agent showed no clear uptake by such cells (E, F).

Conclusion

The annexin A5-functionalized contrast agent presented in this study potentially allows non-invasive assessment of cell types that are considered to significantly contribute to plaque instability, and therefore may be valuable for the diagnostics of atherosclerotic lesion phenotype.



(A-F) Untargeted control micelles and **(G-L)** anxA5-micelles. **(A,G)** CLSM of apoptotic Jurkat cells (blue: nuclei, red: micelles). **(B,C,H,I)** T_1-weighted MR images of abdominal aorta pre- **(B,H)** and 24 hrs post-contrast injection **(C,I)**. **(D,J)** Near-infrared fluorescence images. **(E,F,K,L)** Immunofluorescence images of abdominal aorta tissue sections (blue: nuclei, red: micelles) stained for macrophages **(E,K)** or apoptotic cells **(F,L)** in green.

References: [1] M. J. Davies et al., *Br.Heart J.* 69, 377-381 (1993). [2] F. D. Kolodgie et al., *Am.J.Pathol.* 157, 1259-1268 (2000). [3] Y. Nakashima et al., *Arterioscler.Thromb.* 14, 133-140 (1994).