In Vivo MR Imaging of Acute Thrombus Formation Using a Bimodal Contrast Agent

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Introduction: Thrombus formation plays a central role in several vascular diseases such as myocardial infarction, deep venous thrombosis, and pulmonary embolism. Since the resistance of thrombi against fibrinolytic therapy is achieved within hours, early detection of thrombus formation is vital. During the early stages of thrombus formation, activated factor XIII cross-links α_2-antiplasmin to the fibrin network. Therefore, an α_2-antiplasmin-based contrast agent (CA) may improve patient stratification.

Materials and Methods: A bimodal α_2-antiplasmin-based contrast agent (CA) and a non-specific bimodal control CA (Q3→A3 substitution) were synthesized. Human clots were formed *in vitro* during 90 minutes at 37ºC and exposed to 150µM of the bimodal α_2-antiplasmin-based CA or non-specific bimodal control CA. To determine factor XIII dependence of the α_2-antiplasmin-based CA, dansylcadaverine (factor XIII inhibitor) was added during clot formation and CA exposure. All clots were imaged with a 1.5T whole body MR scanner (Intera, Philips Medical Systems) using a 47 diameter surface radio-frequency coil and two-photon laser scanning microscopy (TPLSM).

For *in vivo* imaging of fresh thrombi, carotid thrombus formation was achieved by 10% $FeCl_3$ treatment. The α_2-antiplasmin-based CA (n=2) or the non-specific bimodal control CA (n=2) was administered intravenously (dose: 5.0 µmol/kg) immediately after the inducement of thrombus formation, followed by *in vivo* MRI using a 7T scanner (Biospec, Bruker) and *ex vivo* TPLSM. Specificity of the bimodal α_2-antiplasmin-based CA for acute thrombus formation was examined by administration of the α_2-antiplasmin-based CA 48 hours after carotid thrombus formation (n=2). Relative signal levels (RSLs) were calculated by dividing mean MR signal intensities from a region of interest (ROI) positioned in the thrombus by the mean MR signal intensities from a ROI positioned in neighboring muscle tissue. In addition, spleen, liver, kidney, heart, lung, and intestines were examined *ex vivo* with TPLSM to determine the biodistribution of the α_2-antiplasmin-based CA.

Results: RSLs at the edge of the *in vitro* formed clots were increased using the α_2-antiplasmin-based CA compared to the non-specific CA. Slight MR signal increase was found in the clot formed in the presence of dansylcadaverine and exposed to the α_2-antiplasmin-based CA. TPLSM showed co-localization of fibrin and the α_2-antiplasmin-based CA, while hardly any co-localization was found using the non-specific bimodal control CA. FXIII blocking in combination with the α_2-antiplasmin-based CA resulted in less fluorescence (red) and less co-localization (figures 1A-C).

In vivo MR imaging using the α_2-antiplasmin-based CA, showed hyperintense signal (RSL: 1.46 ± 0.04) in the $FeCl_3$ treated carotid arteries, while isointense signal was observed in the contralateral carotid arteries. No hyperintense MR signal was found using the non-specific bimodal control CA for fresh thrombus visualization (RSL: 0.94 ± 0.12) as well as using the α_2-antiplasmin-based CA for the visualization of 48 hours old thrombi (RSL: 1.00 ± 0.04). *Ex vivo* TPLSM of the carotid arteries confirmed MRI findings (figures 1D-I). TPLSM of the other excised organs showed that the α_2-antiplasmin-based CA is predominantly cleared through the kidney.

Conclusion: The α_2-antiplasmin-based bimodal CA enables *in vivo* MR imaging of acute thrombus formation. Binding of this CA to the fibrin network is confirmed by TPLSM.

References: 1) Tung et al., ChemBiochem 4: 897-899 (2003), 2) Jaffer et al., Circulation 110: 170-176 (2004).

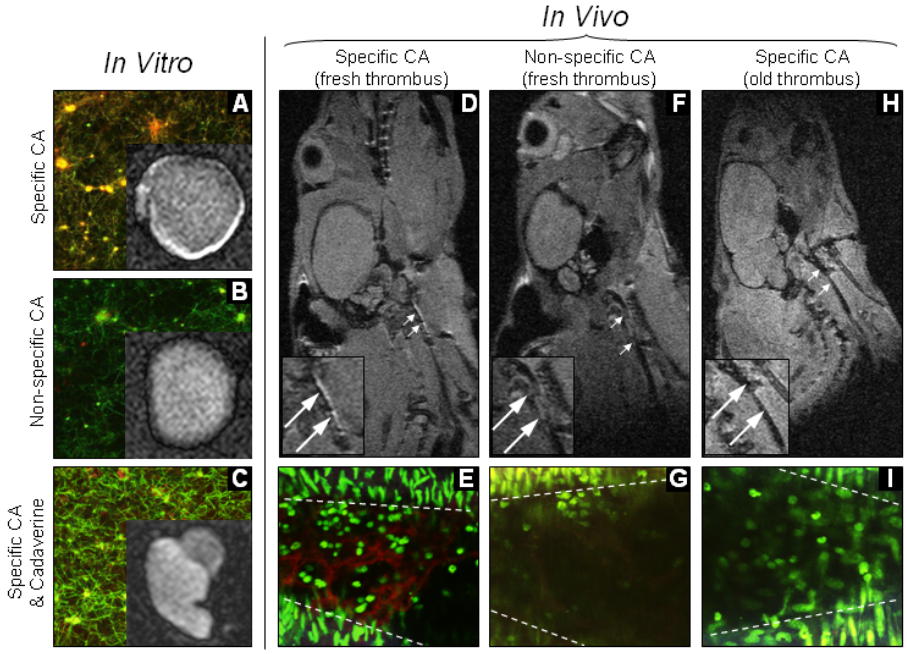

Figure 1: In vitro TPLSM and MRI (inset) of clots with α_2-antiplasmin-based CA (A), non-specific CA (B), and α_2-antiplasmin-based CA in the presence of dansylcadaverine (FXIII inhibitor) (C). Red: Bimodal CA. Green: fibrin network. Yellow: co-localization of bimodal CA and fibrin.
In vivo MRI (D,F, and H) and ex vivo TPLSM (E, G, and I) of fresh (D, E, F, and G) and old (H and I) thrombi in murine carotid arteries after injection of the α_2-antiplasmin-based CA (D,E,H, and I) and the non-specific CA (F and G). Red: Bimodal CA. Green: Nuclear stain. Lumen is located between dotted lines. Elongated cells are smooth muscle cells, spherical cells are leucocytes.