Molecular Targeting of Angiogenic Blood Vessels in Atherosclerotic Plaques with a New Low Molecular Weight Non-Peptidic RGD Mimetic

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Introduction. Atherosclerosis represents the leading cause of death and illness in developed countries and will soon become the pre-eminent health problem worldwide. Recent evidence suggests that neoangiogenesis greatly contributes to the instability of atherosclerotic plaque. The microvessels in atherosclerotic plaque prominently express the integrin alpha(v)-beta(3) [1]. MRI is a promising technology that can provide noninvasive imaging with sub-millimeter resolution and high tissue contrast. In the present work, the noninvasive molecular imaging of plaque associated angiogenesis was assessed with a new low molecular weight non-peptidic RGD mimetic [2]. This alpha(v)-beta(3)-targeted molecule was grafted either to Gd-DTPA or to USPIO and the *in vivo* evaluation was performed on transgenic $ApoE^{-/-}$ mice.

Material and Methods. The RGD mimetic was obtained as described by Sulyok et al [2]. The molecule was grafted onto DTPA by reaction with pSCN-Bz-DTPA (Macrocyclics, Dallas, Tx, USA); DTPA-mimRGD was then complexed with $GdCl_3x6H_2O$. For grafting onto magnetic nanoparticles the dextran coating of USPIO was previously treated with epichlorhydrin. To assess the molecular imaging of atherosclerotic plaques, female C57Bl $apoE^{tm1Unc}$ mice were injected i.v. with one of the following contrast agents: 0.1 mmol/kg b.w. of Gd-DTPA-mimRGD; 60 µmol/kg b.w of USPIO-g-mimRGD or of USPIO. The animals were analyzed at 2 T (Oxford imaging system) and at 4.7 T (Bruker AVANCE-200 system). For the images acquired at 2 T, a half-birdcage RF resonator was adapted to the size of the mouse (25-mm wide and 40-mm length) [3]. The microimaging device was used for the images acquired at 4.7 T. The following MRI protocols were used on the two imaging systems: (A) RARE, TR/TE = 1050/9.6 ms, RARE factor = 4, spatial resolution = 0.09 mm (4.7T, Bruker AVANCE-200); (B) RARE, TR/TE = 3000/20 ms, RARE factor = 4, matrix = 256, spatial resolution = 0.09 mm) (4.7T, Bruker AVANCE-200); (C) fast SE, TR/TE = 2750/25-25 FOV = 23 mm, matrix = 256, spatial resolution = 0.09 mm (2T, Oxford imaging system) ; (D) TOF 3D, TR/TE = 13/3 ms, flip angle = 40°, matrix = 256x128x64, spatial resolution = 0.156x0.156x0.625 mm (2T, Oxford imaging system). Signal intensity (SI) values were measured within several regions of interest in the arterial wall of the aorta. The specific targeting of integrins was tested on Jurkat cells stimulated with phorbol myristate acetate (PMA). Plasma pharmacokinetics of USPIO-g-mimRGD were assessed on Wistar rats, healthy or with an inflammatory pathology known to involve integrin activation (hepatitis induced by concanavalin A).

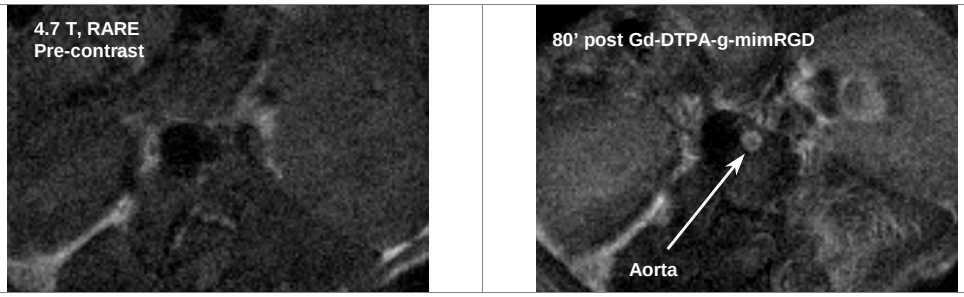

Figure 2. Molecular imaging of atherosclerotic plaque with Gd-DTPA-mimRGD (4.7 T, TR/TE = 1050/9.6 ms, spatial resolution = 0.09 mm).

Results. The images obtained at 4.7 T with Gd-DTPA-mimRGD have shown a striking enhancement (88%) of the signal at the level of atherosclerotic plaque 80 min post-contrast (Figure 1). The SI decrease induced by USPIO-g-mimRGD indicates that the contrast agent accumulates at the level of atherosclerotic plaque early after its administration (30 min) mainly around the blood vessel lumen. The specific interaction of USPIO-g-mimRGD with integrins expressed by Jurkat cells was inhibited by the peptide GRGD by 70%. A prolonged half-life of elimination was observed for USPIO-g-mimRGD (266 min) in pathological conditions associated to integrin expression as compared to healthy state (159 min).

Conclusion. The mimRGD-based contrast agents contribute to the high-resolution *in vivo* molecular imaging and quantification of unstable atherosclerotic lesions. The new contrast agents can find various applications for the MRI detection of angiogenesis-associated pathologies.

References

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