

Stageing of Atherosclerotic Plaques by a Vascular Cell Adhesion Protein-1 Targeted Contrast Agent

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Introduction: Several MRI studies have shown atherosclerosis can be detected *in vivo*, with or without the use of conventional contrast agents. Nevertheless, discrimination of the different plaque components that would predict plaque instability is still very difficult with conventional MRI techniques. One of the most important processes in plaque progression is inflammation, which drives atherosclerotic plaque instability and acute thromboembolic events. Vascular Cell Adhesion Molecule-1 (VCAM-1), a protein that mediates both rolling-type adhesion and firm adhesion, is weakly expressed under baseline conditions but is rapidly induced in activated vascular endothelium as well as in lesional macrophages and smooth muscle cells in later plaque stages. This study aims at visualising and characterising the degree of inflammation in the plaque using VCAM-1 targeted USPIOs as an MRI probe in a mouse model for atherosclerosis.

Methods: A binding peptide for VCAM-1 was identified by phage displayed peptide library screening.¹ Our synthetic peptide or a scrambled variant were covalently conjugated to the carboxyl groups exposed by the bisphosphonate coating of USPIO through their amino-terminal groups. MRI was performed on a 9.4 T vertical Bruker system using a retrospectively gated cine MRI FLASH sequence, acquiring a longitudinal and cross-sectional views of the aortic arch (Fig. 1B). First, contrast agent kinetics were obtained for 24 hours after intravenous injection of USPIO in aged ApoE-/- mice on a Western diet. Secondly, the uptake of VCAM-1 targeted or control USPIO was determined in young ApoE-/- mice on Western diet at 3, 6 and 9 weeks after placement of a constrictive collar around the left carotid artery. After MRI at each time point, the animals were sacrificed for histology.

Results & discussion: We successfully identified a highly specific peptide with nanomolar affinity for human VCAM-1. High resolution MRI performed 1.5 hours after i.v. injection of VCAM-1 targeted USPIOs in aged ApoE-/- mice with advanced plaques showed enhanced uptake of the contrast agent compared to the passive uptake of the scrambled variant (Fig. 1A, 1B). 24 hours after injection, the contrast enhancement was comparable for both groups due to passive uptake of the particles (Fig. 1A). In young ApoE-/- mice with a collar around the carotid artery, uptake of VCAM-1 targeted USPIOs corrected over the plaque area was significantly increased in mice 3 weeks post collar placement. Imaging 6 hours after contrast agent injection showed an increased uptake of the iron particles, particularly in early plaque stages when VCAM-1 expression is highest (3 weeks > 6 weeks > 9 weeks, Fig. 1C). Histology showed co-localisation of VCAM-1 positive endothelial cells and iron deposits in the vessel wall at early plaque stages as well as co-localisation of the USPIO with VCAM-1 positive endothelial cells and lesional macrophages at later plaque stages.

Conclusions: VCAM-1 targeted USPIO show contrast enhancement corresponding to the VCAM-1 expression levels in the plaque. These particles hold great promise for diagnosis and staging, and therapy follow-up of inflammatory plaques in atherosclerosis.

1. Burtea C, Laurent S, Port M, Lancelot E, Ballet S, Rousseaux O, Toubeau G, Vander Elst L, Corot C, Muller RN. Magnetic resonance molecular imaging of vascular cell adhesion molecule-1 expression in inflammatory lesions using a peptide-vectorized paramagnetic imaging probe. *J Med Chem.* 2009 Aug 13;52(15):4725-42.

