Development and validation of a peptide-vectorized superparamagnetic imaging probe designed for the detection of inflammation in atherosclerotic plaque

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Introduction

Atherosclerosis develops in response to vascular injury and involves inflammation and vessel remodeling. VCAM-1 is an important biomarker of various inflammatory disorders having a particular relevance for the pathological process of atherosclerotic disease. Molecularly targeted imaging probes have gained great popularity during the last decade in the context of theranostic strategies. The imaging of VCAM-1 expression has thus been approached with antibodies or peptides conjugated to nuclear, magnetooptical, or ultrasound probes. However, the size of most atherosclerotic lesions is below the spatial resolution of nuclear or ultrasound imaging systems. MRI is the only clinical imaging technique able to attain a spatial resolution in the order of micrometers. Nevertheless, the low sensitivity achieved with magnetic probes constitutes a real challenge for molecular targeting by MRI. Owing to their large NMR efficacy, iron oxide nanoparticles may represent an attractive probe alternative [1, 2]. During our previous work [3], we have identified and validated a VCAM-1-targeted cyclic heptapeptide which was able to detect specifically this adhesion molecule both in a mouse a model of T cell mediated hepatitis, and in atherosclerotic plaque of ApoE-KO mice. In the present work, this peptide was conjugated to USPIO (USPIO-R832), and VCAM-1 binding was first confirmed on HUVEC stimulated with TNF-alpha. Subsequently, USPIO-R832 was evaluated by MRI at 4.7T on ApoE-KO mice, by using T_2 and T_2*-weighted imaging sequences. The ability to bind to atherosclerotic plaque of this molecular imaging probe was furthermore corroborated by histochemistry. The control imaging probe was represented by USPIO vectorized by a non-specific peptide (USPIO-NSP).

Materials and methods

The peptides were conjugated to USPIO as previously described [4]. The size of functionalized USPIO was of ~30 nm, while their r_2 at 60 MHz and 37°C was of 86 s^{-1} mM^{-1} for USPIO-R832 and of 90 s^{-1} mM^{-1} for USPIO-NSP. Female C57Bl ApoE^{tm1unc} mice received a Western diet (0.21% cholesterol) for 3 months prior to the MRI studies. The contrast agents were assessed blindly on 6 mice each at a dose of 100 µmol Fe/kg. Images were acquired (4.7 T Bruker imaging system, Bruker, Ettlingen, Germany) at the level of abdominal aorta with a T_2-weighted RARE sequence (TR/TE = 3000/20 ms, RARE factor = 4, NEX = 4, matrix = 256x256, FOV = 2.3 cm, slice thickness 1 mm, 20 axial slices, spatial resolution = 90 µm) and a T_2*-weighted FLASH imaging protocol (TR/TE = 175/1.88 ms, flip angle = 90°, NEX = 8, matrix = 128x128, FOV = 2.5 cm, slice thickness 1.25 mm, 15 axial slices, spatial resolution = 172 µm). A 3D-TOF sequence (TR/TE = 10/2 ms, flip angle = 20°, NEX = 2, FOV = 4x2x4 cm, matrix = 256x128x64, slice thickness = 1 mm, 60 axial slices, spatial resolution = 156x156x625 µm) was used with the aim to confirm the anatomical location of the aorta in the image slice. SI values for each time point were measured within ROIs drawn manually by using the ImageJ image analysis software in the arterial wall of the abdominal aorta. The standard deviation (SD) of noise was also measured in a region situated out of the animal's image. SI enhancement (%SNR) was then calculated. VCAM-1 expression in atherosclerotic aorta was confirmed by immunohistochemistry, while contrast agents were stained on aortic samples by Perl's Prussian blue staining protocol.

Results

The maximum negative contrast produced by USPIO-R832 on RARE images occurred between ~30 min and 94 min post-injection, probably depending on the level of plaque neovascularization, which influences the diffusion of the contrast agent (Figure 1). This could also be a sign of plaque vulnerability. The plaque surface was measured on images and it was correlated to the level of the plaque enhancement and to the histological observations. In the case of FLASH images, the maximum negative contrast occurred between ~40 min and 80 min post-injection. With the exception of one mouse, the negative contrast was almost absent in the case of USPIO-NSP both on RARE and FLASH images. The histochemistry studies confirmed the MRI results and have shown an extensive VCAM-1 expression, as well as the presence of capillary-like structures that could be of angiogenic nature.

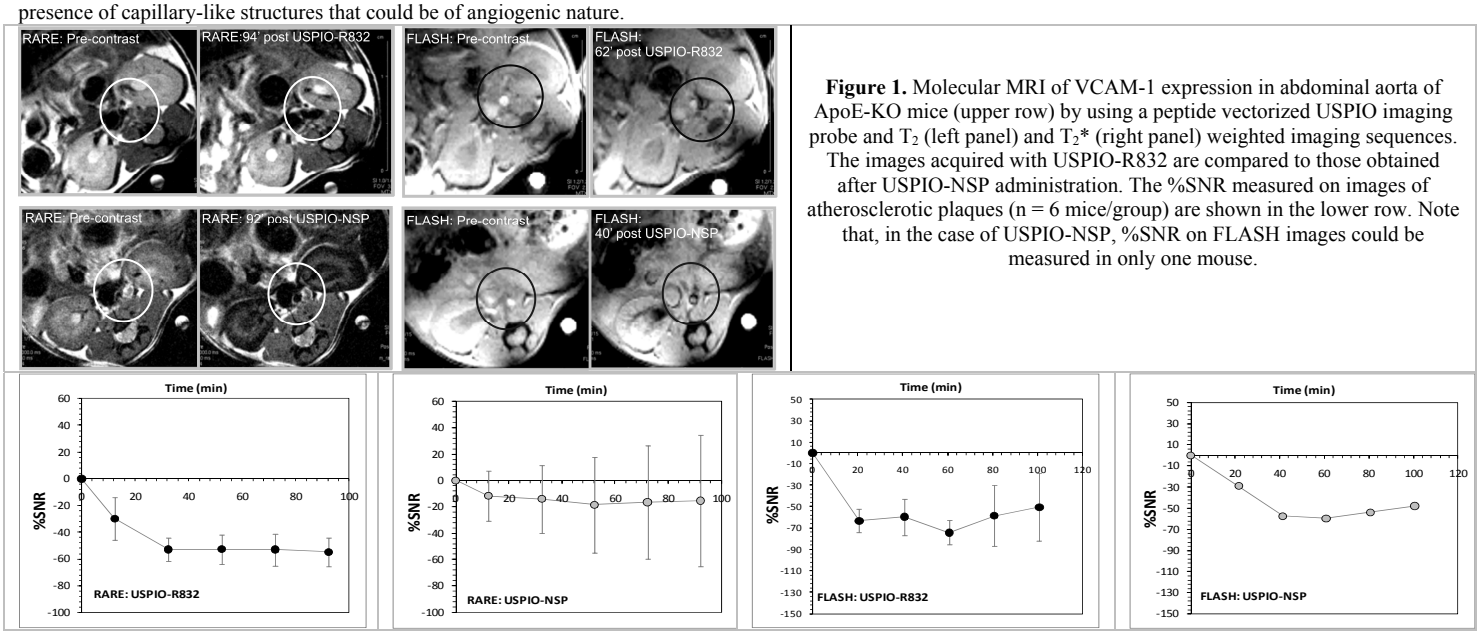

Figure 1. Molecular MRI of VCAM-1 expression in abdominal aorta of ApoE-KO mice (upper row) by using a peptide vectorized USPIO imaging probe and T_2 (left panel) and T_2* (right panel) weighted imaging sequences. The images acquired with USPIO-R832 are compared to those obtained after USPIO-NSP administration. The %SNR measured on images of atherosclerotic plaques (n = 6 mice/group) are shown in the lower row. Note that, in the case of USPIO-NSP, %SNR on FLASH images could be measured in only one mouse.

Conclusions

Our peptide-vectorized, VCAM-1-targeted, superparamagnetic imaging probe seems to be a highly promising tool for atherosclerosis imaging, by considering its ability to attain its target in lower doses and as fast as 30 min after administration. This represents an important progress in comparison with previously developed superparamagnetic agents designed for the same purpose. The lower immunogenic potential and the cost-effectiveness when compared with antibody-conjugated contrast agents represent supplementary arguments for a possible implementation in the clinical practice.

Bibliography

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