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

C. Burtea[1], S. Laurent[1], E. Lancelot[2], O. Rousseaux[2], S. Ballet[2], C. Thirifays[1], M. Port[2], G. Toubeau[3], L. Vander Elst[1], C. Corot[2], and R.N. Muller[1]

[1]General, Organic and Biomedical Chemistry, NMR and Molecular Imaging Laboratory, University of Mons, Mons, Belgium, [2]Research Center, Guerbet, Aulnay-sous-Bois, France, [3]Laboratory of Histology, University of Mons, Mons, Belgium

Carmen.Burtea@umons.ac.be

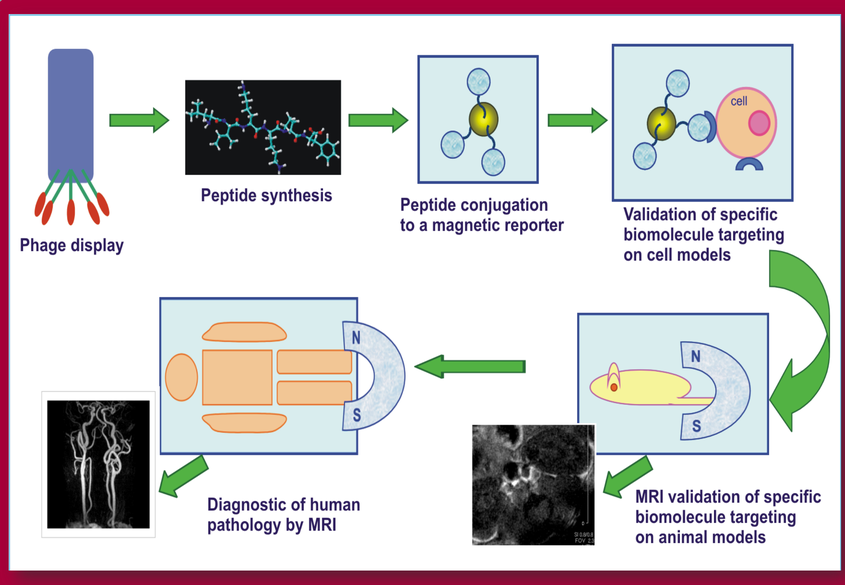
Introduction

- **Atherosclerosis** ➔ vascular injury, inflammation, vessel remodeling
- **VCAM-1** ➔ biomarker of inflammatory disorders and particular relevance for atherosclerotic disease
- **MRI:**
  - The only clinical imaging technique able to attain a spatial resolution in the order of micrometers which is a prerequisite for the imaging of atherosclerotic lesions
  - Improvement of sensitivity by using MR contrast agents with high relaxivity and targeted to atheroma biomarkers ➔ iron oxide nanoparticles may represent an attractive probe alternative owing to their large NMR efficacy [1, 2]

VCAM-1-targeted cyclic heptapeptide identified and validated during our previous work [3]

Aim of the present work :

- The peptide was conjugated to USPIO (USPIO-R832), and the control imaging probe was represented by USPIO vectorized by a non-specific peptide (USPIO-NSP)
- VCAM-1 binding was evaluated by MRI at 4.7T on ApoE-KO mice, by using T_2 and T_2*-weighted imaging sequences
- MRI studies corroborated by histochemistry



Materials and methods

- **Animal model** ➔ female C57Bl ApoE^{tm1unc} mice received a Western diet (0.21% cholesterol) for 3 months prior to the MRI studies.
- **USPIO derivatives:**
  - Peptides conjugated to USPIO as previously described [4]
  - Size of the functionalized USPIO ➔ ~30 nm
  - r_2 at 60 MHz and 37°C ➔ 86 s^{-1} mM^{-1} for USPIO-R832 and of 90 s^{-1} mM^{-1} for USPIO-NSP
  - Assessed blindly on 6 mice each at a dose of 100 µmol Fe/kg
- **MRI:**
  - 4.7 T Bruker imaging system, Bruker, Ettlingen, Germany
  - T_2-weighted RARE sequence ➔ TR/TE = 3000/20 ms, spatial resolution = 90 µm
  - T_2*-weighted FLASH sequence ➔ TR/TE = 175/1.88 ms, flip angle = 90°, spatial resolution = 172 µm
  - 3D-TOF sequence ➔ TR/TE = 10/2 ms, flip angle = 20°, spatial resolution = 156x156x625 µm
  - SI values ➔ measured within ROIs drawn manually by using the ImageJ software. They were drawn on pre-contrast images and then reproduced in post-contrast on the area of signal enhancement around the vessel lumen.
- **Histology** ➔ binding of USPIO-R832 (Perl's-DAB staining), presence of collagen and thrombus (Masson's trichrome staining), of angiogenic blood vessels (VCAM-1 and PECAM-1 staining), macrophages (Mac 1), cholesterol (Sudan IV), cholesterol esters and triglycerides (Nile bleu), and of smooth muscle cells (α-actin staining)

Results

- RARE images ➔ maximum negative contrast produced by USPIO-R832 between 30 min and 94 min post-injection, (Figures 1, 2 and 3).
- FLASH images ➔ maximum negative contrast 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 (Figures 1 and 2).
- The histochemistry studies confirmed the MRI results and are presented in Figures 4 and 5. They have shown an extensive VCAM-1 expression, as well as the presence of capillary-like structures that could be of angiogenic nature. Most of the plaques are type V lesions, i.e. fibroatheroma.

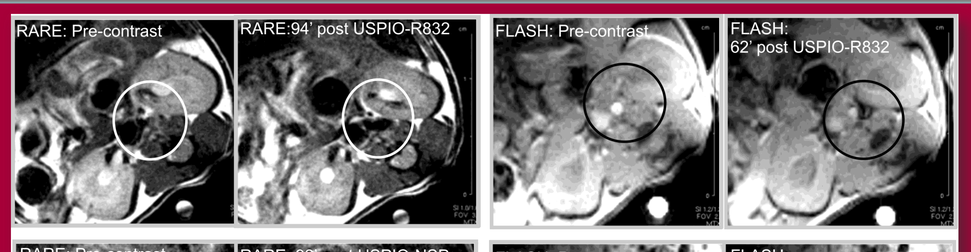


Figure 1. Molecular MRI of VCAM-1 expression in abdominal aorta of ApoE-KO mice (upper row) by using a peptide vectorized USPIO imaging probe and T_2 (left panel) and T_2* (right panel) weighted imaging sequences. The images acquired with USPIO-R832 are compared to those obtained after USPIO-NSP administration.

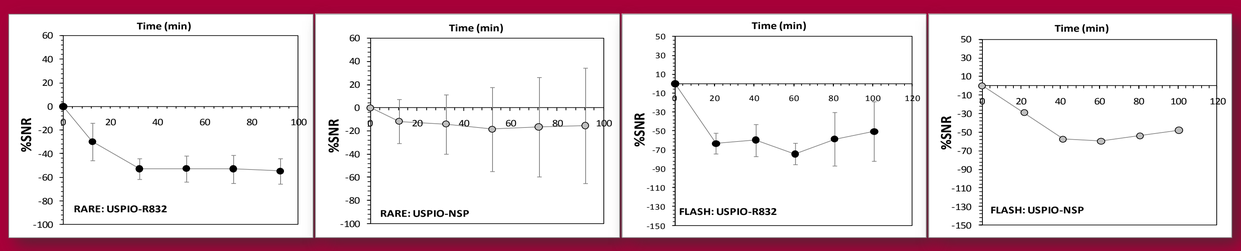
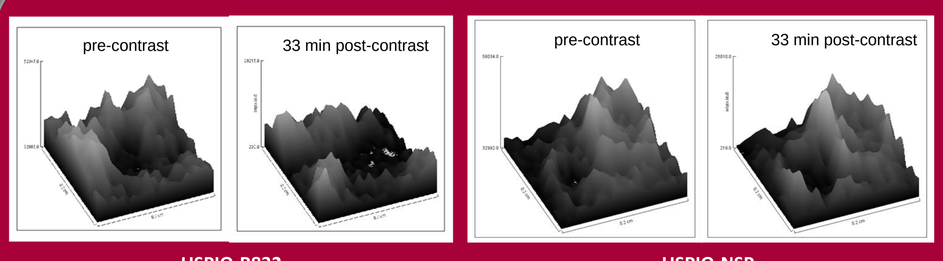


Figure 2. The %SNR measured on images of atherosclerotic plaques (n = 6 mice/group). Note that, in the case of USPIO-NSP, %SNR on FLASH images could be measured in only one mouse.



USPIO-R832 **USPIO-NSP**

Figure 3. The size of atherosclerotic plaques was measured on RARE images and then compared to histologic results. The percentage of black pixels was quantified on pre- and post-contrast images and then compared to Perl's iron staining and VCAM-1 immunostaining on histologic samples (Fig. 5). The plots shown here exemplify the pixel distribution in the plaques as observed by MRI. USPIO-R832 increased the percentage of black (i.e. signal void) pixels in post-contrast image.

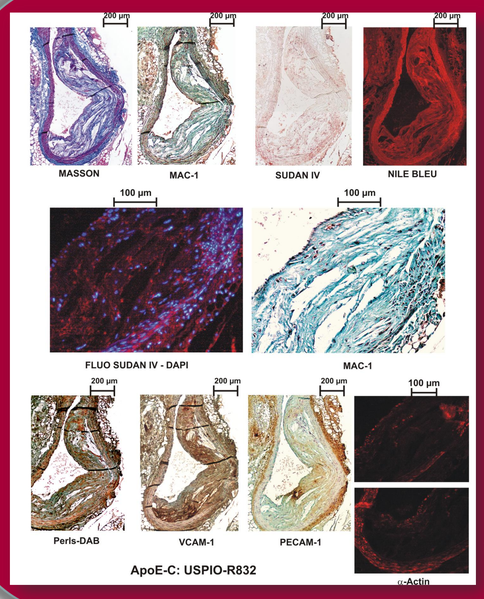
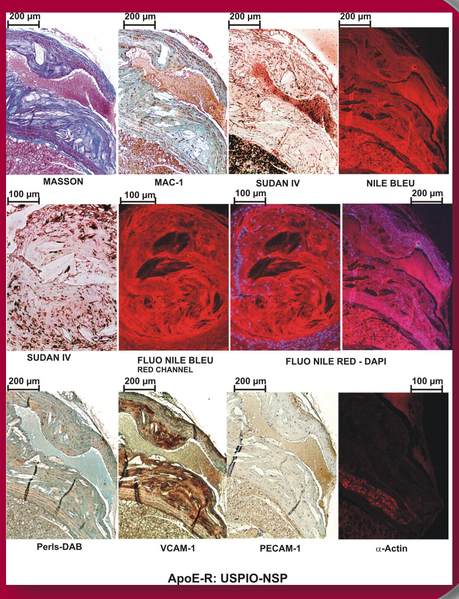
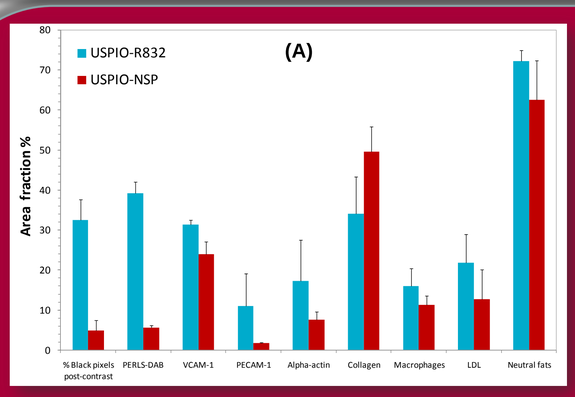
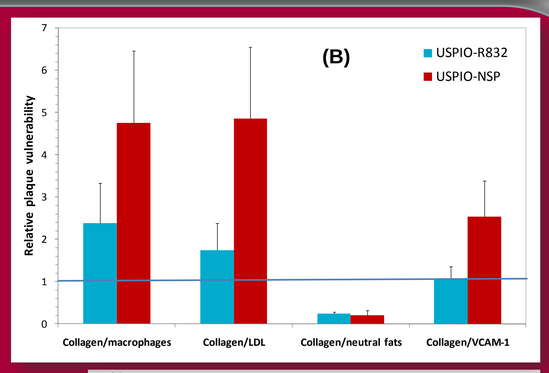


Figure 4. Immunostaining of aorta samples collected from ApoE-KO mice after MRI studies. Histologic pictures were analyzed with ImageJ software to estimate the semi-quantitative distribution (see Fig. 5) of collagen (Masson's trichrome staining), macrophages (Mac 1), cholesterol (Sudan IV), cholesterol esters and triglycerides (Nile bleu), of USPIO-R832 and USPIO-NSP (Perl's-DAB staining), angiogenic blood vessels (VCAM-1 and PECAM-1 staining), and of smooth muscle cells (α-actin staining). The pictures shown here exemplify the staining of aorta samples collected from mice injected with USPIO-R832 (left) or USPIO-NSP (right).







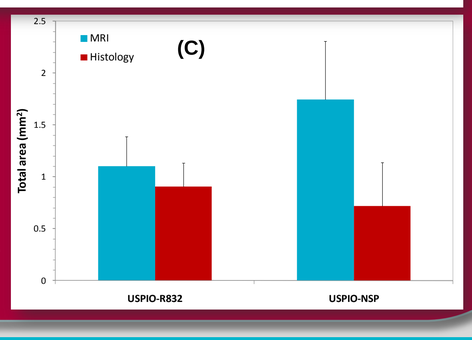


Figure 5. The semiquantitative analysis of histologic pictures shows a correlation between the percentage of black pixels in post-contrast MR images and the distribution of Perl's-DAB and VCAM-1 staining (**A**). Alpha-actin was often distributed in the head of the plaques, indicating their stability. This characteristic was also expressed by the ratio between collagen and macrophages, LDL, neutral fats or VCAM-1 (as a marker of angiogenesis) (**B**). Most of the parameters suggest stable plaques, although they are rich in cholesterol esters (low collagen/neutral fats). The ratio collagen/VCAM-1 shows equilibrate distribution of the two biomarkers indicating again rather stable plaques, which are characteristic for this mouse model. The total plaque areas measured by MRI and histology are almost equivalent.

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.

References

1. Kelly KA, Allport JR, Tsurkas A, Shinde-Patil VR, Josephson L, Weissleder R, Circ Res, 96, 2005, 327–336.
2. McAteer MA, Schneider JE, Ali ZA, Warrick N, Bursill CA, von zur Muhlen C, Greaves DR, Neubauer S, Channon KM, Choudhury RP, Arterioscler Thromb Vasc Biol, 28, 2008, 77–83.
3. Burtea C, Laurent S, Port M, Lancelot E, Ballet S, Rousseaux O, Toubeau G, Vander Elst L, Corot C, Muller RN, J Med Chem, 52, 2009, 4725–4742.
4. Port M, Corot C, Raynal I, Rousseaux O, US Patent 2004/0253181 A1.