Molecular targeting of angiogenic blood vessels in atherosclerotic plaques with a new low molecular weight non-peptidic RGD mimetic

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INTRODUCTION

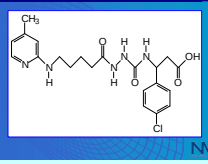
Atherosclerosis represents the leading cause of death and illness in developed countries and will soon become the pre-eminent health problem worldwide. Recent evidence suggests that **neoangiogenesis** greatly contributes to the **instability of atherosclerotic plaque**. The angiogenic blood vessels in the plaque may be particularly fragile and prone to micro-hemorrhage. The microvessels in the adventitia as well as in the plaque prominently express **the integrin alpha(v)-beta(3)**, which is present at the sites of smooth muscle cell accumulation and angiogenesis in atherosclerotic plaques [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

Synthesis of mimRGD and of USPIO-g-mimRGD and Gd-DTPA-mimRGD

The **RGD mimetic** (**Figure 1**) was obtained as described by Sulyok et al [3]. 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 (**USPIO-g-mimRGD**) the dextran coating of USPIO was previously treated with epichlorhydrin. Proton longitudinal (R_1) and transverse (R_2) relaxation rates were measured at 60 MHz and 37°C on a Bruker Minispec mq60 (Bruker, Karlsruhe, Germany).

Figure 1. Structure of the RGD mimetic.



MATERIAL AND METHODS

In vivo MRI evaluation

To assess the **molecular imaging of atherosclerotic plaques**, female C57Bl apoE^{tm1Unc} mice (8 and 18 months old, Charles Rivier Laboratories, Brussels, Belgium & St Germain sur l'Arbresle, France) were injected i.v. through the caudal vein 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 MR images were acquired before and at different time intervals post-contrast up to 130 min, and at 24 h (for USPIO contrast agents). The animals were analyzed at **2 T** (**Oxford & SMIS 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, FOV = 23 mm, spatial resolution = 0.09 mm (4.7T); (B) **RARE**, TR/TE = 3000/20 ms, RARE factor = 4, FOV = 23 mm, matrix = 256, spatial resolution = 0.09 mm) (4.7T); (C) **fast SE**, TR/TE = 2750/25-25 FOV = 23 mm, matrix = 256, spatial resolution = 0.09 mm (2T) ; (D) **TOF 3D**, TR/TE = 13/3 ms, flip angle = 40°, FOV = 40x20x40 mm, matrix = 256x128x64, spatial resolution = 0.156x0.156x0.625 mm (2T). Signal intensity (SI) values for each time delay were measured within several regions of interest in the arterial wall of the abdominal aorta by using the OSIRIS and IMDISP image analysis software. SI values were normalized with respect to a Gd-DTPA/saline solution in a test tube phantom. The data were averaged and the standard error was calculated for each time point. SI enhancement (ΔS/N%) was defined as the ratio of the difference between pre- and post-contrast SI to pre-contrast SI.

Blood pharmacokinetics and validation of the specific interaction with integrins

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 hepatitis induced by concanavalin A (conA).

RESULTS

The in vivo molecular imaging of atherosclerotic plaques: Gd-DTPA-mimRGD

The images obtained at 4.7 T with **Gd-DTPA-mimRGD** have shown an **important enhancement** of the signal at the level of atherosclerotic plaque in abdominal aorta **80 min post-contrast** (**Figure 2**). The evolution of ΔS/N% (**Figure 3**) measured on 10 ROI that cover 8 mm along the abdominal aorta of an ApoE$^{-/-}$ mouse indicates the constant enhancement of SI, which attained a value of 103% 80 min after the administration of Gd-DTPA-mimRGD.

Figure 2. Molecular targeting of atherosclerotic plaque with Gd-DTPA-g-mimRGD

Avance-200 system, 4.7 T, RARE (TR/TE = 1050/9.6 ms, NEX = 4, FOV = 23 mm, slice thickness = 0.8 mm, matrix = 256, spatial resolution = 90 µm)

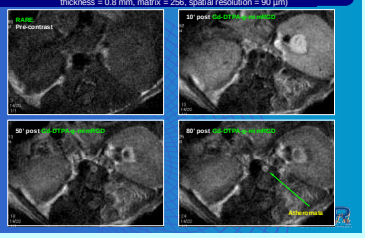
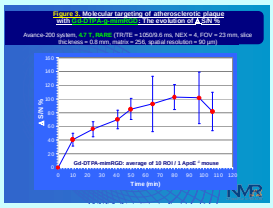


Figure 3. Molecular targeting of atherosclerotic plaque with Gd-DTPA-g-mimRGD: The evolution of ΔS/N%

Avance-200 system, 4.7 T, RARE (TR/TE = 1050/9.6 ms, NEX = 4, FOV = 23 mm, slice thickness = 0.8 mm, matrix = 256, spatial resolution = 90 µm)



RESULTS

The in vivo molecular imaging of atherosclerotic plaques: USPIO-g-mimRGD

Figure 4 shows the images of the atherosclerotic plaque obtained at **2T**, 54 min (FSE T_2) and 160 min (TOF) post USPIO-g-mimRGD. The decrease of SI indicate an **accumulation of USPIO-g-mimRGD** at the level of the atherosclerotic plaque, **mainly around the blood vessel lumen**. The estimation of ΔS/N% on the FSE T_2 images (Figure 5) evidenced that **SI decreased** down to - 44%, 34 min post-contrast, **the lowest level** being observed **130 min post-contrast**. The SI tended to return to the baseline level (-30%) 24 h post-contrast.

Figure 4. Molecular targeting of atherosclerotic plaque with USPIO-g-mimRGD

2 T, Fast SE T2 (FSE, TR/TE = 2500/25-50 ms, FOV = 23 mm, spatial resolution = 90 µm) and 3D TOF (TR/TE = 13/3 ms, FOV = 40x20x40, matrix = 256x128x64, spatial resolution = 156x156x625 µm)

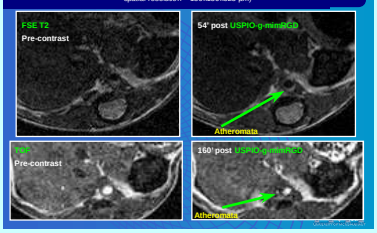
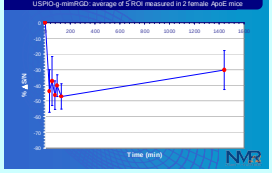


Figure 5. Molecular targeting of atherosclerotic plaque with USPIO-g-mimRGD

The ΔS/N % (2T, SE T_2) in aorta after treatment with USPIO-g-mimRGD: average of 5 ROI measured in 2 female ApoE mice



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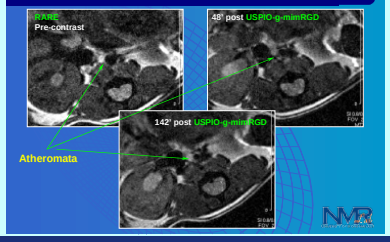
RESULTS

The in vivo molecular imaging of atherosclerotic plaques: USPIO-g-mimRGD

The images obtained at **4.7 T** with RARE protocol (**Figure 6**) are comparable to those obtained at 2 T by using FSE T2 (**Figure 2**), meaning that SI changed around the blood vessel lumen giving the **appearance of the lumen narrowing**.

Figure 6. Molecular targeting of atherosclerotic plaque with USPIO-g-mimRGD

Bruker AVANCE-200 system, 4.7 T, RARE (TR/TE = 3000/20 ms, RARE factor = 4, FOV = 23 mm, slice thickness = 0.8 mm, matrix = 256, spatial resolution = 90 µm)



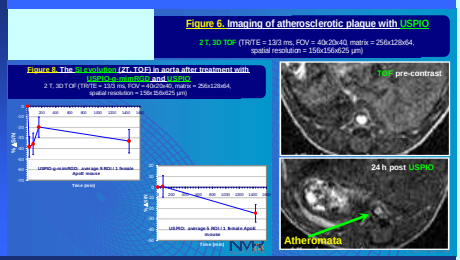
RESULTS

The in vivo imaging of atherosclerotic plaques: USPIO

USPIO (**2T**) did not produce any change of SI at the level of atherosclerotic plaque during the subsequent 2 h post-contrast. **A black strip** was observed on TOF images at the lumenal side of the blood vessel wall **24 h post-contrast** (**Figure 7**). The estimation of **ΔS/N%** on TOF images (**Figure 8**) confirms this type of SI evolution, which is probably correlated to USPIO uptake by macrophages. By the contrary, **USPIO-g-mimRGD** produced the **decrease of ΔS/N%** on TOF images (**Figure 4**) down **to - 39% 24 min post-contrast**.

Figure 6. Imaging of atherosclerotic plaque with USPIO

2 T, 3D TOF (TR/TE = 13/3 ms, FOV = 40x20x40, matrix = 256x128x64, spatial resolution = 156x156x625 µm)



RESULTS

USPIO-g-mimRGD: validation of the specific interaction with integrins and pharmacokinetic characterization

The specific **interaction of USPIO-g-mimRGD with integrins** expressed by Jurkat cells was evaluated by a new technique, C-MALISA [4]. The **K^*_d** of this interaction was estimated to **1.13 x10^{-8} M**. A **diminished blood clearance in pathological conditions** as compared to different controls (healthy animals treated with USPIO-g-mimRGD, animals treated with USPIO) was observed for USPIO-g-mimRGD: prolonged half-life of elimination (266 min); diminished total clearance (0.235 mL/kg/min); diminished VDss (0.066 L/kg).

CONCLUSIONS

The results suggest that integrin targeting with mimRGD-grafted contrast agents contributes to the high-resolution *in vivo* molecular imaging of unstable atherosclerotic lesions. The new compounds could help to the detection of various pathologies associated with angiogenic blood vessel development, such as the cancer.

REFERENCES

[2] Hoshiga M, Alpers CE, Smith LL, Giachelli CM, Schwartz SM, Circulation Research 77, 1995, 1129-1135.

[3] Sulyok GAG Gibson C, Goodman SL, Hölzemann G, Wiesner M, Kessler H, Med Chem, 44, 2001, 1938-1950.

[4] Chaabane L, Canet Soulas E, Contard F, Aly Salah A, Guerrier D, Briguet A, Douek P, Invest Radiol, 38, 2003, 532–538.

[5] Burtea C, Laurent S, Roch A, Vander Elst L, Muller RN, J. Inorg. Biochem. 99, 2005, 1135-1144.