Molecular Imaging of Angiogenic Blood Vessels in Vulnerable Atherosclerotic Plaques with a Mimetic of RGD Peptide Grafted to Gd-DTPA

Carmen Burtea[1], Sophie Laurent[1], Luce Vander Elst[1], Gerard Toubeau [2], Robert N Muller[1]

[1] Department of General, Organic and Biomedical Chemistry, NMR and Molecular Imaging Laboratory,

[2] Department of Histology, University of Mons-Hainaut, 24 Avenue du Champ de Mars B-7000, Mons, Belgium

http://www.umh.ac.be/~nmrlab/index.html

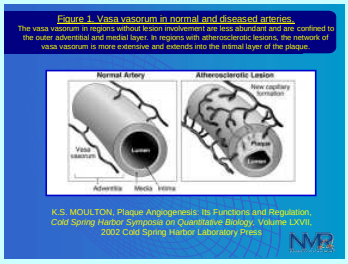
INTRODUCTION

In normal artery:

- microvascular network of vasa vasorum is confined to the adventitia and outer media.

In atherosclerotic artery (Figure 1):

- microvascular networks become more abundant and extend into the intima.



The microvessels in the adventitia as well as in the plaque prominently express the **$\alpha_v\beta_3$ integrin** [4].

The **noninvasive molecular imaging** of plaque-associated **angiogenesis** has been assessed with a low molecular weight **non-peptidic RGD mimetic (mimRGD)** [2] grafted to Gd-DTPA (Gd-DTPA-g-mimRGD). The *in vivo* imaging evaluation has been performed on transgenic ApoE$^{-/-}$ mice, while the pharmacokinetic parameters were determined on Wistar rats.

MATERIAL AND METHODS

Synthesis and physico-chemical characterization of Gd-DTPA-g-mimRGD

The mimRGD was obtained as described by Sulyok [5]. Synthesis was performed on solid support (trityl chloride polystyrol resin) by the Fmoc strategy [6]. This molecule was grafted onto Gd-DTPA compound by reaction between C4-Bz-NCS-DTPA (Macrocyclics, Texas, USA) and the mimetic in aqueous solution (pH=10). DTPA-g-mimRGD was then complexed with $GdCl_3x6H_2O$ to obtain Gd-DTPA-g-mimRGD.

In vivo evaluation: molecular targeting of atherosclerotic plaques by MRI

Animal model of atherosclerosis

Female C57Bl ApoE^{tm1unc} mice, aged ~15 months (n = 10, Charles River, Brussels, Belgium) received a Western diet for three months before the MRI studies. For MRI experiments, the animals were anesthetized with 60 mg/kg b.w., i.p., of Nembutal (Sanofi, Brussels, Belgium). The contrast agents (Gd-DTPA-g-mimRGD and Gd-DTPA) were injected i.v. at a dose of 0.1 mmol/kg. An *in vivo* experiment of competition (n = 4) was performed in the presence of Eu-DTPA-g-mimRGD,. All the images were acquired at the level of abdominal aorta.

MRI equipment and protocols

The experiments were performed on a 200 MHz (4.7 T) Bruker imaging system (Bruker, Karlsruhe, Germany) equipped with a vertical magnet and a micro-imaging device. The images were acquired with the following MRI protocols:

RARE: TR/TE = 1048.5 / 4 ms, RARE factor = 4, NEX = 4, matrix = 256, FOV = 2.3 cm, slice thickness 0.8 mm, spatial resolution = 90 µm.

MSME: TR/TE = 695.8/8.9 ms, NEX = 2, FOV = 2.3x2.3 cm, matrix = 256x256, slice thickness = 1 mm, spatial resolution = 90x90 µm.

3DTOF was used with the aim to confirm the anatomical localization of the aorta in the image slice: TR/TE = 10/2 ms, FA = 20°, NEX = 2, FOV = 4x2x4 cm, matrix = 256x128x64, slice thickness = 1 mm, spatial resolution = 156x156x625 µm.

In vivo evaluation: plasma pharmacokinetics

Plasma pharmacokinetics were assessed on Wistar rats (Harlan, Horst, The Netherlands). Gadolinium content of the blood samples (collected through the carotid artery) was determined by relaxometry (37°C, 60 MHz, Bruker Minispec). The following pharmacokinetic parameters were calculated: the elimination half-life ($T_{e1/2}$), the steady-state volume of distribution (VD_{ss}) and the total clearance (Cl_{tot}).

Immunohistochemistry

The presence of atherosclerotic plaques and of microvascular network was assessed on aortas collected from mice after MRI evaluation. CD31 was detected with rat anti-mouse PCAM-1 biotin-conjugated monoclonal antibody (Chemicon International).

RESULTS

Molecular targeting of atherosclerotic plaques by MRI

The specific interaction of mimRGD with integrins was previously assessed after grafting to USPIO (USPIO-g-mimRGD) [6]. The K^*_d for integrins was of $1.13x10^{-8}$ M in activated state and of $4.60x10^{-7}$ M in non-activated state; the pre-incubation with linear peptide GRGD inhibited the interaction at the receptor sites by 70%.

Figure 2:

- Axial slices of abdominal aorta before and after the administration of Gd-DTPA-mimRGD.
- The external structures of the aortic wall (probably tunica media and adventitia) are strogly enhanced 10 min post-contrast; this enhancement persists up to 55 min.
- The more profound layers (toward the aortic lumen) of the aortic wall (possibly tunica media and intima) can also be distinguished. The aortic lumen seems to be restrained and distorted.

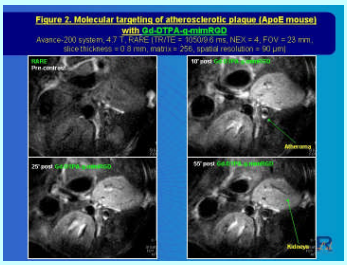


Figure 3:

- The same mouse was injected with Gd-DTPA and compared with Gd-DTPA-mimRGD.
- The enhancement produced by Gd-DTPA is rather diffuse and the aortic wall is not clearly outlined.

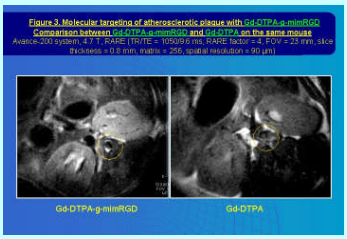
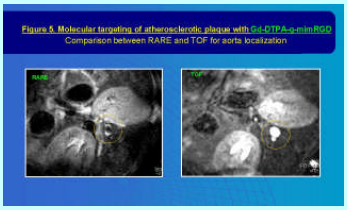


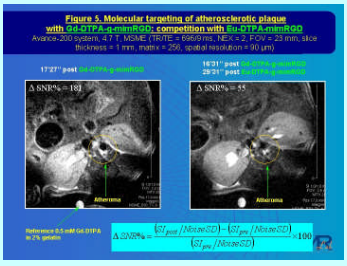
Figure 4:

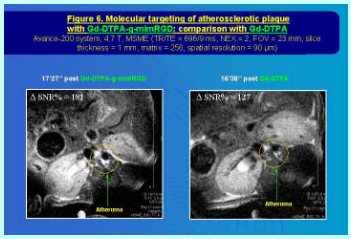
The aorta localization was possible due to the MRI protocol 3DTOF, which has also evidenced the backward enhancement of the aortic wall (a clear spot toward the spinal cord).



Figures 5 and 6:

- in vivo competition experiment to validate the specific integrin targeting at the level of atherosclerotic plaques.
- The results are compared to contrast enhancement in non-competing conditions (Figure 6) and to that produced by the non-specific contrast agent Gd-DTPA (Figure 7).



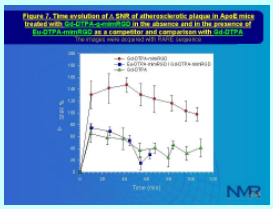


The signal enhancement of the aortic wall obtained in competing or non-specific conditions is 40 – 90 % lower than with Gd-DTPA-g-mimRGD. This effect is either global, or restricted to a certain area of the aortic wall. This means that a certain fraction of the contrast agent (specific or non-specific) is free to circulate into the microvascular network of the aortic wall, which explains the relative signal enhancement produced even by the non-specific compound, Gd-DTPA. Even low, such a non-specific enhancement could represent a potential drawback for the specific diagnosis of atherosclerotic disease in this particular case. In clinical practice, this could be solved by subtracting the images obtained with non-specific compound from the ones produced by the specifically targeted contrast agent. In this way, the pathologic areas expressing the targeted receptor could be delineated.

Figure 7:

Gd-DTPA-g-mimRGD induced the maximum signal enhancement 42 min post-contrast (147 %).

With Eu-DTPA-g-mimRGD, ΔSNR does not surpass the level produced by Gd-DTPA and ranges between 75 % and 15 %.

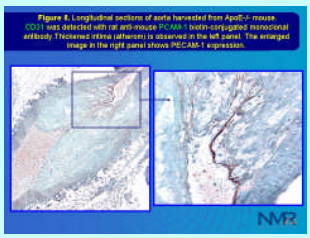


Pharmacokinetic characterization

A diminished blood clearance was observed for Gd-DTPA-g-mimRGD: prolonged $T_{e1/2}$ (61 min, compared to 15 min for Gd-DTPA); diminished Cl_{tot} (3.6 mL/kg/min, compared to 8.7 mL/kg/min for Gd-DTPA).

Immunohistochemistry

The presence of atherosclerotic plaques was confirmed on histologic sections (**Figure 8**). The thickened intima was positive for PECAM-1 expression.



CONCLUSION

The non-peptide RGD mimetic grafted to Gd-DTPA (Gd-DTPA-g-mimRGD) was designed for the targeting of $\alpha_v\beta_3$ integrin, which is an adhesion molecule over-expressed on angiogenic blood vessels in various pathologies, such as atherosclerosis and cancer. The new contrast agent could greatly contribute to the high-resolution *in vivo* molecular imaging methods, aimed at the localization and quantification of unstable atherosclerotic lesions.

REFERENCES

[1] Libby P, Nature 420 (2002) 868 – 874.
[2] Moulton KS et al, Circulation, 99, 1999, 1726-1732.
[3] Moulton KS et al, PNAS, 100 (8), 2003, 4736 – 4741.
[4] Hoshiga M et al, Circ Res, 1995;77:1129-1135
[5] Sulyok GA et al, J Med Chem, 44 (12), 2001, 1938 – 1950.
[6] Burtea C, Laurent S, Roch A, Vander Elst L, Muller RN, J Inorg Biochem, 99(5), 2005, 1135 – 1144.