Ultrasmall particle of iron oxide – RGD peptidomimetic conjugate as novel MRI contrast agent

S. Laurent[1], C. Burtéa[1], V. Rerat[2], J. Marchand-Brynaert[2], L. Vander Elst[1], and R. N. Muller[1]
[1]Université de Mons, Mons, Belgium, [2]Université catholique de Louvain, Louvain-la-Neuve, Belgium

Introduction

RGD peptidomimetics conjugated to ^{111}In- and ^{89}Y-DOTA complexes, and to Gd-DTPA complex, have been recently developed to target the $\alpha_v\beta_3$ receptor.[1] In this work, we describe the grafting of a new RGD peptidomimetic on ultrasmall particles of iron oxide (USPIO) coated with 3,3'-*bis*(phosphonate)propionic acid in the context of magnetic resonance molecular imaging.The grafting rates have been evaluated by X-ray photoelectron spectroscopy (XPS) while the USPIO-g-Mimic have been characterized by photon correlation spectroscopy (PCS), magnetometry and NMR relaxometry. Finally their ability to target leukemic cells was also analyzed.

Materials and methods

The structure of our functional magnetic contrast agent targeting $\alpha_v\beta_3$-displaying cells is shown in Scheme 1: the carboxylated iron particle is connected to the RGD peptidomimetic *via* an oligoethylene glycol spacer; amide linkages are used to assemble the three building blocks. The Nuclear Magnetic Relaxation Dispersion (NMRD) profiles were recorded on a Fast Field Cycling Relaxometer (Stelar, Italy) over a range of magnetic field extending from 0.24 mT to 0.24 T. Additional longitudinal (R_1) and transverse (R_2) relaxation rate measurements were obtained at 0.47 T and 1.41 T on Minispec PC 120 and MQ 60 spin analyzers respectively (Bruker, Germany). The magnetization measurements were performed on a known amount of ferrofluid using a vibrating sample magnetometer (VSM-NUOVO MOLSPIN, UK). Hydrodynamic size measurement was carried out by photon correlation spectroscopy (PCS) on a Malvern system (Zetasizer Nanoseries ZEN 3600).

Results

The peptidometic used in this study stems from previous work.[2] USPIO-g-Mimic (figure 1) and USPIO-g-GRGDS were prepared from nanoparticles with carboxylated groups on surface as described by Port et al.[3] The modified particles were analyzed by XPS for determining their surface atomic composition. The derivatization rates recorded by XPS analysis were about 3-4%, corresponding to 2-2.4 molecules of $\alpha_v\beta_3$ ligand per particle.

Physico-chemical properties of the novel USPIO conjugates were measured to control the particle size and the magnetic characteristics: hydrodynamic mean diameter was determined by dynamic light scattering (21-37 nm); from magnetometric and relaxometric profiles, the magnetization at saturation (Ms=54-66 A.m² / kg) and Fe microcrystal radius (4.7-5.2 nm) were obtained.

The relaxometric properties of the USPIO-g-Mimic (r_1 = 35.0 $mM^{-1}s^{-1}$, r_2 = 72.1 $mM^{-1}s^{-1}$ (20 MHz, 37°C) and r_1 = 17.5 $mM^{-1}s^{-1}$, r_2 = 73.4 $mM^{-1}s^{-1}$ (60 MHz, 37°C)) did not differ much from those of the USPIO-g-GRGDS (r_1 = 37.2 $mM^{-1}s^{-1}$, r_2 = 72.3 $mM^{-1}s^{-1}$ (20 MHz, 37°C) and r_1 = 19.0 $mM^{-1}s^{-1}$, r_2 = 71.8 $mM^{-1}s^{-1}$ (60 MHz, 37°C)). It can be thus concluded that the grafting of GRGDS peptide or of mimetic does not change significatively the relaxometric properties of the nanoparticle.

The magnetic labelling of cells expressing $\alpha_v\beta_3$ integrin was performed with Jurkat T lymphocytes. These leukemic cells are known to express the $\alpha_v\beta_3$ receptor under stimulation with phorbol 12-myristate 13-acetate (PMA, 50 ng/mL, 3h, 37°C, 5% CO_2). Cells (1.5 x 10^6 cells/mL), stimulated or not (negative control), were incubated in USPIO-conjugate solutions (0.5 mM) during 2 h at 25 °C. After washing out the excess of particles, cells were seeded in a gelatine matrix for measuring T_2 (CPMG pulse sequence, Minispec Mq-60, 60 MHz, 37 °C). The efficiency of USPIO capture by cells is determined by the R_2^{norm} values (where $R_2 = 1/T_2$, and R_2^{norm} is the normalized R_2 calculated by subtracting the R_2 of cells free of USPIO from the R_2 of cells incubated with iron oxide nanoparticles), the highest values corresponding to the best cell targeting. Results (table 1) showed a clear difference between activated cells (PMA) and non activated ones (CONtrol). The native particles gave some unspecific cell adhesion, independently of their activation state. After conjugation to the RGD peptidomimetic, the particles were more efficiently trapped by the activated cells (increase of 229% as compared to CON cells; $p<0.05$). This targeting effect was also visible in the case of particles conjugated to the reference peptide, but the peptide appeared less active than the peptidomimetic probably because the reaction of conjugation to USPIO could have altered the affinity of the reference peptide for the target.

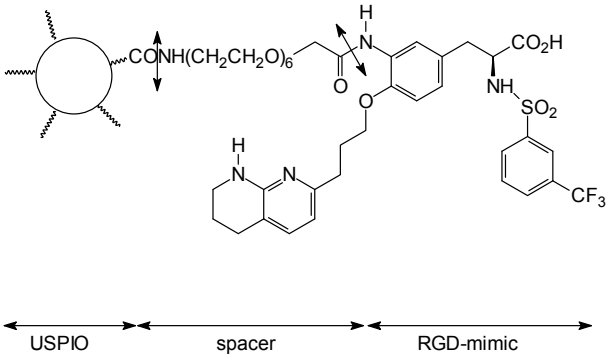

Figure 1: Structure of USPIO-g-mimic

Table 1: Targeting Jurkat cells with USPIO conjugates

Contrast agent	PMA	CONTROL
	R_2^{norm} (s^{-1})	
USPIO	4.0±0.5	3.2±0.3
USPIO-*g*-Mimic	6.5±1.4 ($p<0.05$)	2.8±0.2
USPIO-*g*-GRGDS	5.2±2.7	3.0±1.0

Conclusions

With its nanomolar binding affinity, the new RGD peptidomimetic appears as a good vector for contrast agents. For sensitive MRI applications, USPIO are very promising if made stealty to circumvent capture by the phagocytic cells of the reticuloendothelial system. After optimization of the conjugation yield (e.g. >2.4 molecules/particle) and adequate coating with an adequate stealth layer, this novel USPIO-RGD peptidomimetic could thus assist tumor cell detection by MRI..

References

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