Multimodal imaging probes for vascular endothelial growth factor receptor

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INTRODUCTION

Atherosclerosis is manifested by a formation of lipid-rich plaques within the walls of large arteries, and if untreated, results in myocardial infarction, chronic stable angina and stroke. Non-invasive detection of atherosclerosis could be potentially performed by using conventional methods using superparamagnetic or paramagnetic contrast agents combined with MRI, which permits non-invasive vascular imaging with sub-millimeter resolution and high tissue contrast. However, since these contrast agents non-specifically enhance the MR imaging signal, adequate information about the biological activity of atherosclerotic plaques cannot be obtained. Thus, detecting molecules expressed on the surface of activated macrophage is a considerably more challenging for detecting early stage of atherosclerosis. To target the macrophages in atherosclerosis, the present study had a focus on: 1) vascular endothelial growth factor receptor-1 (Flt-1) as a biomarker 2) to improve the uptake, we used particles 30-50 nm in diameter; 3) optical imaging properties of the micelles.

METHODS

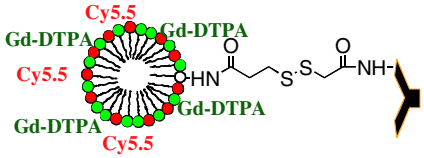
Nanosized micelles were prepared using two lipids, 1,2-Dioleoyl-*sn*-Glycero-3-Phosphoethanolamine (DOPE) and L-phosphatdylcholine (Egg PC). Three types of DOPE derivative, DOPE-DTPA, DOPE-SS-2Py and DOPE-Cy5.5, were prepared by covalently binding with diethylenetriaminepentaacetic acid dianhydride (DTPA), N-succinimidyl 3-(2-pyridyldithio) propionate (SPDP) and fluorescent dye (Cy 5.5), respectively. DOPE-DTPA-Gd was further modified by chelating DOPE-DTPA with $GdCl_3$. Various lipid micelles were prepared by PC with altering composition ratio of DOPE-DTPA, DOPE-SS-2Py and DOPE-Cy5.5. The micelles were conjugated to thioacetylated monoclonal vascular endothelial growth factor receptor antibody (anti-Flt1) which was modified by reacting with N-succinimidyl S-acetylthioacetate (SATA). After completely removing free anti-Flt1, physicochemical properties of synthetic micelles were characterized by laser light scattering, atomic force microscopy and elemental analysis prior to in vitro use.

J744A1 macrophage-like cells were used in this study as a model and the cells were activated by treating with lipopolysaccharide (LPS). Cell-binding profiles were quantitatively analyzed using flow cytometry. For MR and optical imaging, approximately 2×10^6 cells were used by suspending the cells in 100 □l of 0.1% agarose gel and solified at 4 °C.

MRI was performed at a clinical 1.5 T superconducting magnet (GE Medical systems, Milwaukee, WI) using a 3 inch inner diameter surface coil with spin-echo sequences (TR 400 ms, TE 13 ms, 1.0 mm imaging slices). Optical images were obtained using a custom-built instrument equipped with a CCD camera.

RESULTS AND DISCUSSION

Various nano-micelles were prepared and their physicochemical properties were listed at Table1. The initial micelles had hydrodynamic radii of 40 nm or less, however, their sizes were increased by conjugation with 3±2 antibody per each micelle. The relaxivity was increased with the increase of molecular mass. The relaxivity of micelle-aFlt1 was in the range 6.8-7.1 ($mM^{-1}sec^{-1}$) per Gd^{+3}.



Schematic structure of synthetic micellar targeted contrast agent.

Table 1. Properties of synthetic paramagnetic micelles and their components

	MW	Hydrodynamic radius (nm)	Number of Gd^{+3} /	Cy5.5 ($mmolL^{-1}$)	R1 ($mM^{-1}sec^{-1}$)
MA	115-120kD	36 ± 4	6 ± 1	1.3	5.7
MB	116-118kD	37 ± 5	4 ± 1	1.6	5.6
MC	110-120kD	38 ± 7	2 ± 1	2.1	5.1
MA-aFlt1	50kD-55kD	41 ± 5	6 ± 1	1.2	7.4
MB-aFlt1	50kD-55kD	40 ± 4	4 ± 1	1.2	7.1
MC-aFlt1	50kD-55kD	42 ± 6	2 ± 1	1.4	6.8
Gd-DTPA	0.5kD		1	-	4.1
Gd-DTPA-DOPE	1.2kD		1		4.8

DOPE-DTPA-Gd: DOPE-Cy5.5: DOPE-SPDP (MA = 3:1:2:4, MB = 2:2:2:4, MC = = 1:3:2:4)

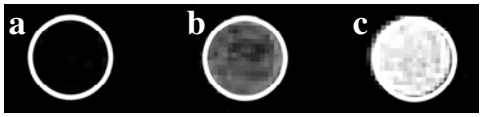


Fig. 1. T1-weighted 1.5TMRI of J744A cells. a) Non-activated control cells, incubated with MA-aFlt1 and activated control cells incubated with b) MA and c) MA-Flt1.

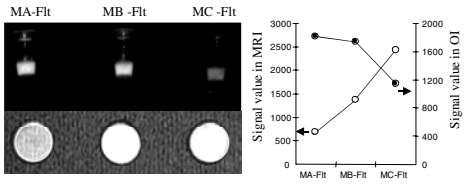


Fig. 2. Comparative uptake of Gd and Cy5.5 in LPS-activated J744A cells. Top- fluorescent image and bottom-T1 weight MRI of J744A cell pellets after incubating with MA, MB, MC.

Non-activated J744A1 macrophage cells didn't show the uptake of micelles or micelle-aFlt1 conjugates. However, after activating of the cells with LPS, 12% of the cells showed the uptake of micelles. In contrast, micelle-aFlt1 conjugates were taken up by 92% of cells (confirmed by confocal microscopy and FACS). Although J744A cells are known to have macrophage-like properties, anti-Flt1 attached to micelles provided high affinity to only the activated cells. Similar results were obtained by using T1-weighted MRI (Fig. 1). There was no detectible signal enhancement in non-activated J744A cells. Therefore, micelle-aFlt1 conjugates had high affinity to activated macrophage-like cells. Both fluorescent and T1-weighted MR images of activated J744A cells by LPS were obtained by using the same sample (Fig. 2). The signal intensity of cell pellets in both images was dependent on concentrations of Gd^{+3} or Cy5, respectively. The final concentration of Gd^{+3} or Cy5.5 in cells could be controlled by altering Gd/Cy composition ratio.

CONCLUSION

We demonstrated the utility of well-known Flt-1 overexpression on the surface of activated macrophages and applied this to paramagnetic micelle targeted to Flt-1. The new synthetic imaging probes were highly specific to Flt-1 expressed on activated macrophage cells and could be detected by optical imaging system as well as MRI. The current study shows that micelle-Flt1 could be potentially used for macrophage-targeted multimodal imaging of early stage of atherosclerosis and localization of inflammatory processes.

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