A Liposomal System for Contrast-enhanced MR Imaging of Molecular Targets

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Introduction

In recent years, magnetic resonance imaging([1]) (MRI) has emerged as one of the leading non-invasive *in vivo* imaging modalities and also holds great promise for the imaging of molecular markers of disease. MRI, however, suffers from limited resolution and specificity, compared to optical and traditional staining techniques. Compared to nuclear tracer methods like PET or SPECT, MRI has low inherent sensitivity([1]). A way to overcome these limitations is to use targeted contrast agents, which can be directed to a molecular entity of interest (e.g. an endothelial cell surface receptor that is over-expressed as a consequence of the disease process). The low inherent sensitivity of MRI requires highly effective contrast agents. This can be achieved by the use of colloidal particles, which contain large amounts of Gd-DTPA([2]). The goal of this study was to develop fluorescently labeled paramagnetic liposomes with a poly(ethylene glycol) (PEG) coating to provide long circulation *in vivo* and with a targeting ligand coupled to the distal end of the PEG-chains to introduce specificity (fig. 1). We tested the liposomes *in vitro* on HUVECs that were treated with TNFα to upregulate the expression of E-selectin and were able to detect the expression of this molecular marker with MRI and fluorescence microscopy.

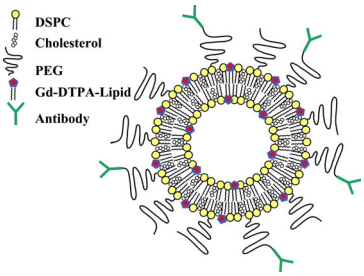

Figure 1. Paramagnetic immunoliposome

Materials and Methods

DSPC/Cholesterol/Gd-DTPA-bis(sterylamide)/PEG-DSPE/Mal-PEG-DSPE (molar ratio 1.25/1/0.6/.075/.075) and Rhodamine-PE (0.1 mol%) containing liposomes were prepared by lipid film hydration. The liposomes were sized by extrusion and the size and size distribution were determined by dynamic laser light scattering. In order to test the stability of the liposomes, the size and the release of calcein from the aqueous interior of the liposomes were determined upon incubation in 10% calf serum at 37° C for 24 hours. Antibodies were coupled to the distal ends of the Maleimide-PEG-chains as previously described([3]). HUVECs were stimulated with TNFα (100 ng/ml) to induce E-selectin expression and incubated with the Gd-DTPA-bis(sterylamide) containing immunoliposomes (1000 nmol/ml) (II). Several control experiments were done (fig. 2A): Stimulated HUVECs with bare liposomes (I), non stimulated HUVECs and immunoliposomes (III), and non incubated HUVECs (IV). T1-Relaxivity and MRI experiments were performed on PCR cups with collected HUVECs (10^6 cells/cup) on a 6.3 Tesla MRI scanner.

Results

The size of the liposomes was 120.3+/-1.5 nm before coupling of the antibody and 148.8+/-1.0 nm after coupling of the antibody. The liposome size before and after incubation in 10% calf serum at 37° C for 24 hours did not differ. The release of calcein after 24 hours was approximitaly 5%. These data demonstrate that this liposomal formulation is stable.

Stimulated HUVECs showed a strong increase in the uptake of immunoliposomes as can been seen from the fluorescence microscopy images (fig. 2B). This uptake resulted in a shortening of T1 and T2 as can be seen from the T1-map and T2-map in figures 2C and 2D. The values for T1 and T2 for the test system (fig. 2A: II) and the different controls (2a: I, III, IV) are listed in table 1.

Table 1 (n=3)	T1 (ms)	T2 (ms)
I HUVECs, immunoliposomes	2156 ± 130	93 ± 14
II HUVECs, TNFα, immunoliposomes	457 ± 62	14 ± 2
III HUVECs, TNFα, bare liposomes	2135 ± 84	104 ± 11
IV HUVECs	2412 ± 124	106 ± 15

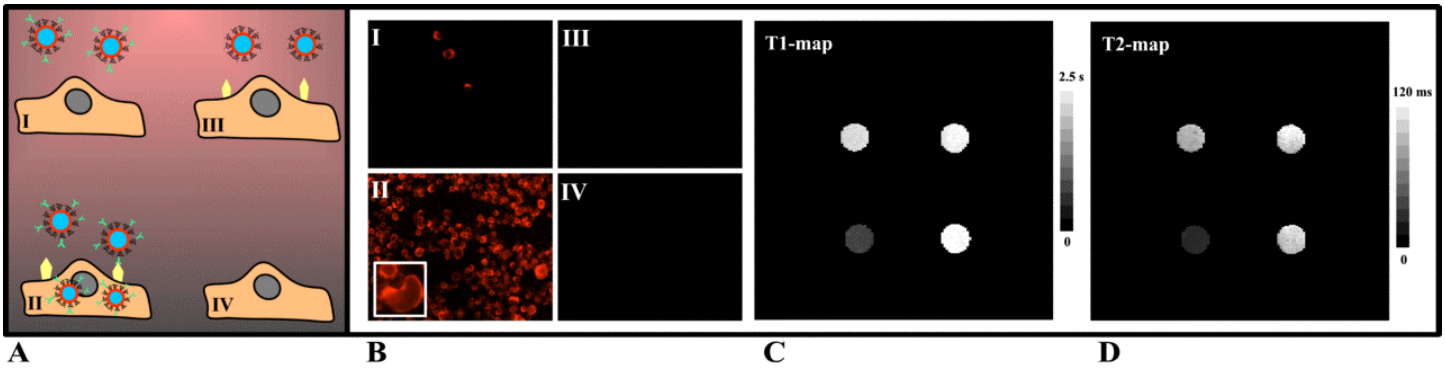

Figure 2. **(A)** Schematic representation of the test system (II) and the controls (I,III,IV). **(B)** Fluorescence images of cells from the setup in A. **(C)** T1-map and **(D)** T2-map of pellets of 10^6 cells (10 ul) from the setup in A. All MR Images: FOV 3 x 3 cm.

Discussion

These results demonstrate that this novel MR contrast agent may potentially serve as a useful diagnostic tool to investigate disease processes. The system is very flexible, can prepared in sizes ranging from 80 nm up to 500 nm, is coated with PEG, and is stable upon incubation in 10% calf serum, which makes it applicable *in vivo*. The liposomes can be targeted by the coupling of an antibody. In a similar manner peptides can also be coupled to the PEG chains([4]) and contribute to the specificity of this contrast agent. Inflammation, angiogenesis and apoptosis are examples of processes, which we aim to detect with these liposomes in a non-invasive way using MRI.

References

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