Imaging targeted delivery of liposomes to tumor vasculature

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Introduction: Receptors specific to tumor vasculature present opportunities to target carriers containing therapeutic and imaging agents. The discovery of new ligands, antibodies and peptides that specifically bind to markers specific to the tumor vasculature is important in developing these strategies. Phage display is a promising approach to identify tumor specific peptides [1]. Such peptides can be used in combination with liposomes as vehicles for the delivery of therapeutic, diagnostic and analytical agents to the tumor vasculature [2]. Here we have examined the potential of a new peptide for targeting multifunctional liposomes against breast cancer vasculature. We selected cationic liposomes because they are a class of lipid vesicles with demonstrated potential in systemic gene delivery [3]. The incorporation of magnetic resonance imaging (MRI) contrast and optical agents within these liposomes allowed *in vivo* visualization of the dynamics of accumulation at the target [4]. The targeting and binding properties of these liposomes were examined in an MDA-MB-231 human breast cancer xenograft model by *in vivo* MRI and optical microscopy.

Materials and Methods: Cationic liposomes with the base formulation DOTAP:DOPE:DOPE-MPB (1:0.95:0.05 molar ratio) were prepared by lipid hydration and extrusion through a polycarbonate membrane with 100 nm pores. A small amount (0.2 mol %) of Rhodamine B-DOPE was included to allow for high-resolution fluorescence microscopy to confirm localization. Gd-DTPA-bis(oleylamide) (Gd-BOA) (60 mol %) was added to the base formulation in place of the DOTAP to monitor delivery of peptide by MR imaging. A FITC-labeled targeting peptide was coupled to the liposome via the DOPE-MPB. We also used scrambled FITC – labeled peptide as a control that was coupled to the liposomes also via maleimide chemistry. Prior to each *in vivo* experiment the T_1 value of the liposome preparation was examined. For the *in vivo* experiments we used the MDA-MB-231 breast cancer model in female SCID mice. A total of 250 µl of liposomes suspension (10 mg/ml of total lipids) was injected intravenously (IV) and mice were imaged with a Xenogen IVIS 200 optical imaging device and MRI before and following the IV injection for 3, 6, 24, 48 and 72 h. MRI experiments were performed on a Bruker BioSpec 4.7 T horizontal bore magnet. Multi-slice T_1 weighted images were acquired with a multislice - spin echo (MSME) sequence. Quantitative multi-slice T_1 maps were generated from relaxation delays of 100, 500, 1000 and 7000 ms using a modified SNAPSHOT FLASH sequence. Following imaging animals were sacrificed and organs were sectioned. The biodistribution of targeted liposomes and liposomes with control peptide was examined with optical imaging. T_1 values of the organs were also obtained using an inversion recovery sequence on a Bruker 11.7T spectrometer. The localization of liposomes within the tumor was determined by fluorescence microscopy.

Results: The liposomes with targeting and control peptides have sizes in the range of 70-120 nm according to dynamic light scattering measurements. The presence of peptides on the surface of liposomes did not alter the hydrodynamic radius. The T_1 value of the liposome suspension was in the range of 40-45 ms prior to IV injection and they were stable for two-three weeks at 4°C. Following IV injection of targeted and control liposomes mice were imaged using MR for 3, 6, 24, 48 and 72 h. A high fraction of targeted liposomes accumulated in the tumor by 3 h post injection according to the T_1-weighted images and T_1 values from the imaged tumor slices. At 6 h we observed maximum intensity in the T_1-weighted images and T_1 values remained almost constant up to 72 h. In contrast, changes in tumor T_1 in mice injected with liposomes and control peptide were not significant at 3 h. By 6 h there was a small change in T_1 values, which persisted until 24 h. However, by 48 h liposomes with control peptide were found to clear from the circulation (Fig. 1 and 2). Similar differences in targeted and control peptide liposomes were observed in tumor sections imaged *ex-vivo* using the Xenogen optical imaging device. Results from the biodistribution using T_1 values obtained from tumors and organs showed significantly different T_1 values for the tumors with targeted liposomes compare to the control. We also observed an accumulation of liposomes in the liver, spleen and intestine for targeted and control peptide liposomes.

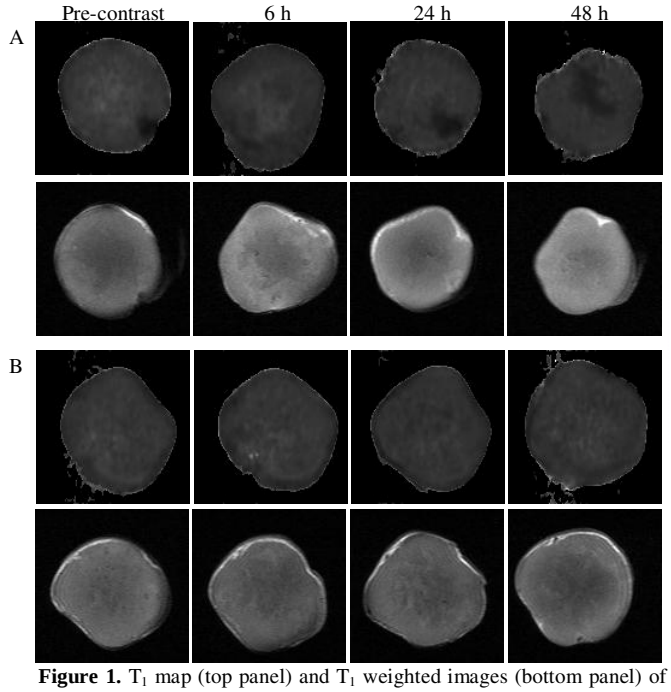

Figure 1. T_1 map (top panel) and T_1 weighted images (bottom panel) of representative slices from tumor with (A) targeting and (B) control liposomes.

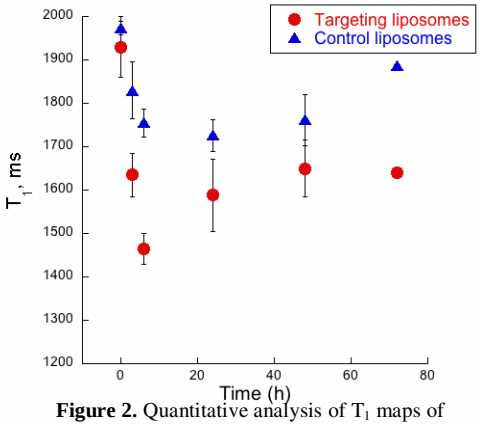

Figure 2. Quantitative analysis of T_1 maps of tumors from *in vivo* experiment

Discussion:

Liposomes with targeted peptide showed a significant difference in tumor accumulation compared to liposomes with control peptide, as observed with optical and MR imaging. In particular, the analysis of T_1 maps demonstrated a significant drop in T_1 values at 6h after IV administration for liposomes coupled with the targeted peptide. The T_1 value for the targeted liposomes also remained lower at 72 h (Fig. 2) compared to the control peptide; these data are consistent with the biodistribution observed using optical imaging. Co-localization of the targeted liposomes with a vascular marker, CD34, confirms the binding properties of the peptide to the tumor vasculature. These results suggest that the newly identified peptide may provide a means to deliver therapeutic and imaging-contrast cargo to breast cancers.

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