Magnetic Resonance Imaging of Delivery Multifunctional Liposomes with Novel Peptide Ligand Targeted to Breast Cancer Vasculature

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Introduction

Tumor growth depends on the formation of new blood vessels to maintain a sufficient supply of oxygen and nutrients. The surfaces of newly formed blood vessels express specific molecules that are not commonly found in the normal endothelium. Therefore one of the approaches in developing new anti cancer drugs and markers that would target nascent tumors is based on the discovery of new ligands, antibodies and peptides that specifically bind to markers specific to the tumor vasculature. Phage display has been shown to be a promising approach to identifying tumor specific peptides [1]. Liposomes are a promising vehicle for the delivery of therapeutic, diagnostic and analytical agents to the developing vasculature in cancer [2]. Here we examine the potential of a new peptide for targeting multifunctional liposomes 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 allows in vivo visualization to assess the dynamics of accumulation at the target [4]. The targeting of these liposomes was examined in the MDA-MB-231 human breast cancer xenograft model by in vivo MR imaging 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 polycarbonate membrane with 100 nm pore size. A small amount (0.2 %) of Rhodamine B-DOPE was included to allow for high-resolution fluorescence microscopy to confirm localization. Gd-DTPA-bis(oleylamide) (Gd-BOA) was added to the base formulation in place of varying fractions of the DOTAP to monitor delivery of peptide by MR imaging. The targeting peptide was coupled to the liposome via the DOPE-MPB or by using carbodiimide activated DOPE. For the *in vivo* experiments we used a breast cancer xenograft model in female SCID mice which were inoculated with 2×10^6 MDA-MB-231 cells in 50 µl of Hanks balanced salt solution subcutaneously in the mammary fat pad. When tumor sizes reached a size of 200-400 mm^3, 200 µl of liposomes suspension was injected intravenously (IV) and mice were imaged with MRI before and following the IV injection. The distribution and localization of targeted liposomes and liposomes only, used as a control, were also examined at different time points using optical imaging. MRI experiments were performed on a Bruker BioSpec 4.7 Tesla horizontal bore magnet. Multi-slice T1 weighted images were acquired with a multislice - spin echo (MSME) sequence. Quantitative multi-slice T1 maps with relaxation delays 100, 500, 1000 and 7000 ms were obtained with a modified SNAPSHOT FLASH sequence.

Results

The transmission electron microscopy and dynamic light scattering of the liposomes preparations revealed a normal liposomal morphology with an average radius of 70-80 nm. Following IV injections of liposomes with targeted peptide and liposomes only (control) MR images were recorded for at least 90 min (Fig. 1). At 30 min liposomes with the targeted peptide exhibit significant binding in the peripheral region of the tumor while the control sample remained similar to the baseline (pre-contrast). At 90 min the histogram of T1 map shows a decrease for targeted liposomes (Fig. 2, left). The liposome only sample does not show any significant change up to 60 min (Fig 2, right). Targeted liposomes were detected by correlated fluorescence microscopy in MDA-MB-231 tumor sections within 60 min of IV administration. Some targeted liposomes also co-localized with a vascular marker (CD34) [5].

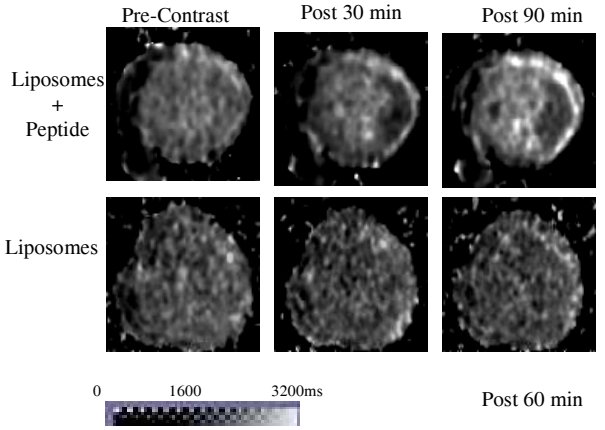

Figure 1. T1 map of representative slice from tumor with targeted peptide coupled to liposomes and liposomes only

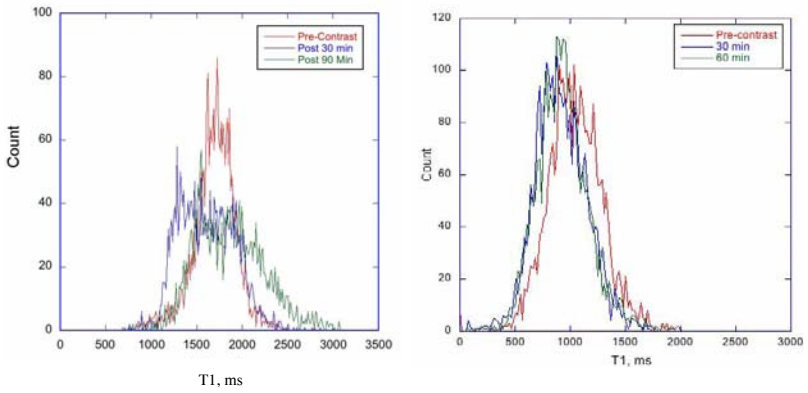

Figure 2. Histograms of the T1 maps of liposomes with targeted peptide (left) and liposomes only (right)

Discussion

The liposomes with targeted peptide showed significant difference in the binding behavior, compared to non-targeted liposomes, as observed with optical and MR imaging. In particular, the analysis of T1 maps demonstrated a significant shift in T1 values at 30 min after intravenous injection for the liposome coupled with the targeted peptide. The stain with CD34 confirms the specificity of the peptide as a vascular targeting agent. These results suggest that the newly identified peptide may provide a means to deliver therapeutic and imaging-contrast cargo to breast cancers.

References

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