In vivo Detection of Lymphatic delivery of liposomes using DIACEST MRI labeling

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INTRODUCTION

Effective cancer chemotherapy depends on the efficient delivery of anticancer drugs to their target at a sufficient concentration, which can be accomplished using passively targeted nano-size carriers.[1] Lymphatic nodes are sites for many diseases including cancer metastasis, and it is important to manufacture drug delivery systems with the ability to target them. Among the documented payload systems, liposomes are one of the most highly developed delivery particles that are approved clinically for the treatment of cancer. Liposomes are known to accumulate in lymph nodes upon subcutaneous injection,[2] and labeling of liposomes with MRI contrast agents to track their fate *in-vivo* therefore has great clinical relevance for imaging cancer metastases. Previously, paramagnetic (Gd-based) agents have been used to image such liposomal systems.[3] In this study, we demonstrate that another MRI contrast mechanism, diamagnetic Chemical Exchange Saturation Transfer (DIACEST), can also be used to visualize liposome accumulation in regional lymph nodes in mice. Contrary to paramagnetic agents, DIACEST contrast can be turned on and off without distorting MRI image contrast.

METHODS AND MATERIALS

L-arginine (L-arg, Sigma-Aldrich) was dissolved in 10 mM PBS to 14 mM and 100mM. CEST liposomes composed of phosphatidylcholine (PtdCho), cholesterol (1:1 mole ratio) and nitrobenzoxodiazol (NBD, a fluorescence dye) labeled lipids (1.8 total mole %) were formed by the modified extended hydration method[4] using a starting solution containing 100 mg/mL of L-arg. Subsequent solutions used to prepare the liposomes came from this stock. After liposomes were extruded, unencapsulated L-arg was removed by dialysis using a 250 [5]kD cutoff dialysis tubing (Spectrum Laboratories Inc). The size and concentration of the liposomes were then measured using dynamic light scattering (Nanosizer, 90ZS, Malvern Instruments) and fluorescence (Victor V, Perkin Elmer). The final stock of liposomes had a particle concentration of 30-100 nM with average liposome sizes varying from 100-400 nm. C57Bl6 mice were injected with dying cancer cells to produce an immuno-responsive enlargement of the popliteal lymph nodes 1 week prior to the injection of lipsome.[6] 30 ul solutions containing 30 nM radioisotope labeled liposomes (containing ^{111}InCl in the interior [5]) at different size that were injected subcutaneously in mouse footpad to determine the pharmacokinetics in C57B16 mice using small animal SPECT. A same condition was used to inject liposome of 100 nm size in mice for CEST MRI study . After the in vivo MRI study, the popliteal lymph nodes were excised and the fluorescence intensity was measured using a Xenogen imager.

All MRI images were acquired at 310K using an 11.7T Bruker Avance system equipped with a 20 mm sawtooth RF coil. A modified RARE (TR=6.0 sec, effective TE= 4.3 ms, RARE factor =16, slice thickness=0.5 mm, and NA=2) including a magnetization transfer (MT) module (one CW pulse, (w_1) = 3.6 μT (150 Hz), 4sec) was used to acquire CEST weighted images from -3ppm to 3ppm (step=0.2ppm) around the water resonance (0ppm). The exact water center frequency for the CEST images was measured using the WAter Saturation Shift Reference (WASSR) method[7] modified for use at high field. In brief, The WASSR pre-scan was conducted prior to CEST imaging with identical image readout (except TR=2 sec) using a short, weak saturation pulse (t_{sat}= 200 ms, w_1 =0.5 μT (21.3 Hz)) sweeping from -2ppm to 2 ppm (0.1ppm step). All data processing was performed using custom-written scripts in Matlab. Arginine-based CEST contrast at +2 ppm from water was computed. A CNR threshold of 2 allowed us to get rid of 'artificial' contrast due to random noise.

RESULTS AND DISCUSSION

To determine the optimal liposome size for lymph node accumulation, we used small animal SPECT/CT of labeled liposomes (Fig. 2A). Only the right footpad was injected with liposome so that the left lymph node could serve as a 'control'. The SPECT study on 5 mice revealed that a liposome size of 100 nm would allow a high accumulation of liposome in popliteal lymph nodes 24 hours post-injection, with larger sizes reducing the uptake. These conditions therefore were used in our DIACEST MRI study. The DIACEST image (Fig. 2B) clearly showed that the right popliteal lymph node was highlighted by a strong CEST signal, while the left side barely showed any CEST. Because DIACEST liposomes were also labeled with a fluorescent chromophore (NBD), the amount of liposome inside the lymph nodes could be semi-quantitatively determined using fluorescent imaging of excised popliteal lymph nodes. A good correlation with the CEST signal (MTR_{asym}) was found, confirming that CEST signal came from the presence of liposomes.

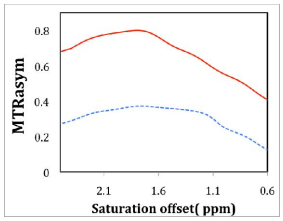

Figure 1. CEST properties of 14 mM L-arginine (pH 7.3, blue dash line) and 30 nM highly sensitive DIACEST liposome filled with L-arginine(red solid line).

CONCLUSION

We demonstrated the first highly sensitive DIACEST liposome system with lymphatic accumulation ability by filling a liposome with a natural compound, L-ariginine. The accumulation of liposome in the popliteal lymph node was visualized using CEST MRI, which provides a practical way to monitor the lymphatic delivery of anticancer drugs by these particles. Because L-arginine is a natural compound, these results could lead to the development of clinically applicable CEST particles for improving cancer chemotherapy.

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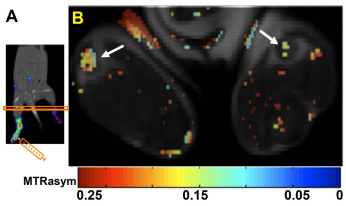

Figure 2. In vivo imaging of DIACEST liposome in mouse popliteal lymph node. A) a SPECT image after 24 hours of injection to footpad, B) the hybrid image of CEST contrast map with T2w anatomical MRI image, the area indicated by white arrows are right and left popliteal lymph nodes.

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