CEST imaging of particle-based therapy for cervical tumors

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Introduction

Nanoparticle-based drug delivery has great potential to improve the efficacy of therapies, in particularly for treatment of cancer[1]. Controlled release of therapeutics from a locally-applied nanoparticle system may provide a new option for early stage cervical cancer compared to the current standard of care involving surgery or radiation therapy[2,3]. We are interested in using Chemical Exchange Saturation Transfer (CEST), a molecular MRI contrast mechanism, to monitor the vaginal drug delivery of nanoparticles for local treatment of cervical tumors by co-encapsulating diamagnetic contrast agents and drugs within liposomes. The goal is to image the distribution of these particles with good temporal and spatial resolution and to indirectly assess the retention of particles over the course of treatment. To demonstrate this, we have collected both *in vitro* and *in vivo* images with PEG-coated liposomes that have frequency offsets further from water (at 5 ppm) than our previous designs[4]. The integration of CEST and pegylated liposomal nanoparticles provides a non-invasive and quantitative way to evaluate the performance of these particles *in vivo* in preclinical studies.

Method CEST liposome preparation and their stability: Drug-containing liposomes were prepared with the poly(ethylene) glycol (PEG) concentration varied systematically using the thin film hydration method[4,5]. In brief, 25 mg of lipid dissolved in chloroform was dried, with the resultant thin film hydrated using 1 ml barbituric acid (BA) to form multilamellar vesicles. The mixture was then annealed at 55 °C, sonicated, and subsequently extruded through stacked polycarbonate filters. Doxorubicin (DOX) was then loaded into the liposomes as described previously[5]. The size (z-average) and heterogeneity in size (polydispersity index, PDI) were measured to indicate the stability of liposomes. Animal Preparation: C57BL/6 mice were given Depo-Provera (3 mg/mouse) by subcutaneous injection on the right flank 4 days prior to tumor inoculation. Luciferase expressing C3.34 cells were then implanted into the intravaginal cavity of mice, and bioluminescence imaging was used to monitor the growth of the tumor. CEST imaging: Mice were anesthetized using isoflurane and positioned in a 11.7T horizontal bore Bruker Biospec scanner, and were imaged before and after intravaginal administration of 20 ul of BA/DOX PEGylated liposomes 3 weeks after inoculation. CEST images were acquired through collection of two sets of saturation images, a water saturation shift referencing (WASSR)[6] set for B_0 mapping and a CEST data set for characterizing contrast. For the WASSR images, the saturation parameters were t_{sat}=500 ms, B_1=0.5 uT, TR=1.5 sec with saturation offset incremented from -1 to +1ppm with respect to water in 0.1ppm steps, while for the CEST images: t_{sat}=3 sec, B_1=4.7 uT, TR=5 sec, with offset incremented from -6 to +6 ppm (0.3 ppm steps) with a fat suppression pulse. The acquisition parameters were: TR=5.0 sec, effective TE= 21.6 ms, RARE factor=8. The CEST images were acquired every 30 min after the liposome administration up to 2 hrs. Data Analysis: MR images were processed using custom-written Matlab scripts with the CEST contrast quantified by calculating the asymmetry in the magnetization transfer ratio (MTR_{asym}) using $MTR_{asym}=(S^{-\Delta\omega}-S^{\Delta\omega})/S_0$ for NH protons at $\Delta\omega$ = 5ppm.

Results and discussion We developed new liposomes loaded with BA (CEST imaging agent) and DOX (chemotherapeutic) (Fig 1a). The *in vitro* CEST contrast for these liposomes with 0%, 5%, 10% and 20% PEG was 24%, 13%, 15% and 13% respectively at 5 ppm (Fig 1b). These particles were stable over 2 days *in vitro* (Fig 1c) with no significant change in the size and PDI. Among these liposomes, we selected formulations with more than 10% PEG for MR imaging due to their homogeneity as measured by PDI (Fig 1c). We imaged PEGylated liposomes administered into the vaginas of mice bearing the C3.34 tumors. The CEST liposomes could be visualized using MRI after local administration and their distribution could be traced over 2 hrs (Fig 2a). The liposomes were located close to the opening of the vaginal cavity (ROI1), and seemed to stay in a distal region (ROI2) inside the vagina (Fig 2b). The contrast in ROI1 and ROI2 increased right after administration and over the first 60 min. Interestingly, the contrast in ROI2 was much higher than that in ROI1 at 90 min post-administration, and it is higher than that of pre-administration. This indicated that liposomes could be retained inside the vagina for at least 2 hrs.

Conclusion Liposomes with both CEST agent and drug were developed for imaging a particle-based therapy of cervical tumors. Measuring the CEST contrast at 5 ppm, we showed that we can image the spatial distribution of the particles after administration and over time *in vivo*.

References [1] Peer D et al. Nat. Nanotechnol. 2007;2(12):751-760. [2] Tang BC et al. PNAS 2009;106(46):19268-19273. [3] Jensen PT et al. Cancer 2004;100(1):97-106. [4] Liu G et al. Magn. Reson. Med. 2011, *In Press*; [5] Tagami T et al. J Cont. Release 2011;152:303-309; [6] Kim M et al. Magn. Reson. Med. 2009;61(6):1441-1450. **Supported by NIH grants R01EB015031 and R01EB015032**

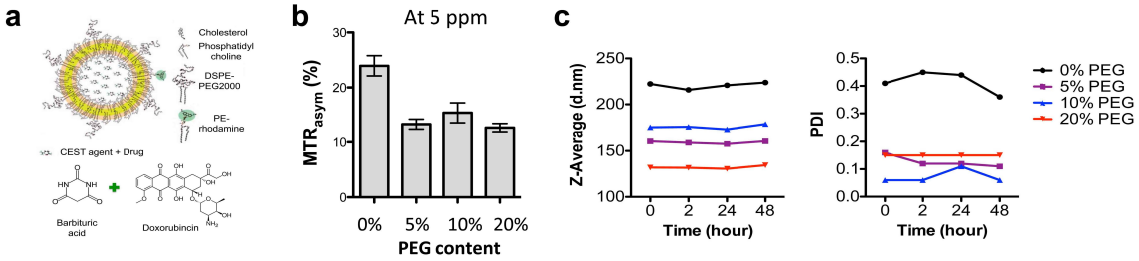

Fig. 1 a) A schematic diagram to show the compositions of BA/DOX liposomes; b) CEST contrast at 5 ppm saturation frequency for liposomes with 0%, 5%,10% and 20% poly(ethylene) glycol (PEG) incorporated in the shell; c) size (Z-Average) and heterogeneity in size (polydispersity index, PDI) of liposomes over 2 days.

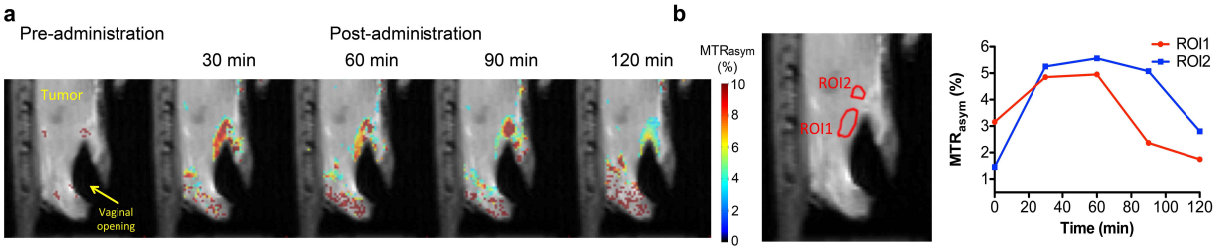

Fig. 2 a) CEST/T2w overlay images at 5 ppm of a cervical tumor bearing mouse before and after administration of the BA/DOX liposomes with 20% PEG; b) region of interest (ROI) analysis using two regions, one (ROI1) close to and a second (ROI2) distal from the site of administration.