Development of CEST liposomes for monitoring nanoparticle-based cancer therapies

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Targeted audience: Investigators who are interested in using MRI to monitor nanoparticle-based therapy.

Introduction: Nanoparticle-based drug delivery has great potential for improving the efficacy of chemotherapy in the treatment of cancer (1). Controlled release of therapeutics from nanoparticles may provide a new alternative for cancer treatments e.g. using vascular-active agents (2). However, the clinical translation of nanoparticle-based chemotherapy has experienced challenges; one of them is the lack of tools to evaluate the biodistribution and pharmacokinetics of the nanoparticle-based chemotherapeutics in cancer patients during the course of treatment. Here, we aim to develop theranostic nanoparticles (i.e. providing therapy and diagnostics) with good temporal and spatial resolution based on diamagnetic Chemical Exchange Saturation Transfer (diaCEST) - a molecular MRI contrast mechanism allowing the use of non-metallic and biocompatible contrast agents. Towards this goal, we have developed a stable formulation of theranostic PEG-coated liposomes that have a frequency offset further from water (at 5 ppm) than our previous design (3), and assessed their distribution and retention in a murine colon cancer model. The integration of CEST agents with nanoparticles provides a non-invasive, quantitative and potentially translatable way to probe nano-chemotherapeutics in tumors.

Method diaCEST liposome (DL) preparation: Drug-containing liposomes were prepared with the poly(ethylene) glycol (PEG) concentration varied systematically using the thin film hydration method. 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 55-65 °C, sonicated, and subsequently extruded through stacked polycarbonate filters (3). Doxorubicin (DOX) was then loaded into the liposomes remotely. Animal Preparation: Five million CT26 cells were injected subcutaneously into the right flank of a mouse and allowed to grow for ~10 days prior to MRI. CEST imaging: Mice were anesthetized using isoflurane and positioned in a 11.7T horizontal bore Bruker Biospec scanner, and were imaged before and 24 h after intravenous administration of 100 ul of BA/DOX PEGylated liposomes. CEST images were acquired through collection of two sets of saturation images, a water saturation shift referencing (WASSR) 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 before and 24 h after the liposome administration. 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 theranostic DLs loaded with both CEST imaging agent (BA) and chemotherapeutic agent (DOX) (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). Among these liposomes, we also studied the formulations with 10% PEG and 30%, 50% and 70% cholesterol (Fig. 1c). In order to determine if these formulations would be stable in the course of imaging (typically <24 h), we compared the CEST contrast among different formulation at 24 h after dialysis (Fig. 2a) and selected the one with the highest contrast and with ~20% BA retained in the liposomes 24 h after dialysis (Fig. 2b; H5C_3P) for *in vivo* study. We imaged mice bearing colon tumors before and 24 h after i.v. injection of PEGylated liposomes. The CEST liposomes could be visualized using MRI after administration and their distribution can be assessed after 24 h of administration (Fig 2c) with a MTR_{asym} of ~6%. This indicated that liposomes were retained inside the tumor for up to 24 h, and detected after systemic i.v. administration.

Conclusion Theranostic DLs with both CEST agent and drug were developed for imaging a particle-based chemotherapy of colon tumors. Measuring the CEST contrast at 5 ppm provides information of spatial distribution of the particles after administration and over a period of 24-h *in vivo*. We are now performing further experiments to compare animals with and without treatment with the vascular-active agent.

References (1) Peer D et al. Nat. Nanotechnol. 2007;2(12):751-760. (2) Qiao Y et al. Oncotarget 2011;2:59-68. (3) Liu G et al. Magn. Reson. Med. 2011, *In Press*. **Supported by NIH grants R01EB015031 and R01EB015032**

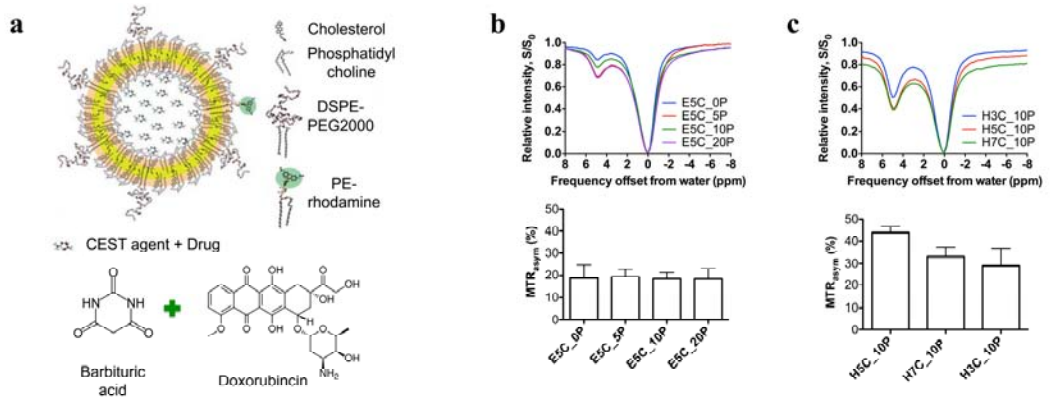

Fig. 1 **a**) Schem showing the compositions of BA/DOX liposomes; **b**) CEST contrast at 5 ppm offset frequency for liposomes with 0%, 5%,10% and 20% poly(ethylene) glycol (PEG) incorporated in the shell; **c**) with 30%, 50% and 70% cholesterol.

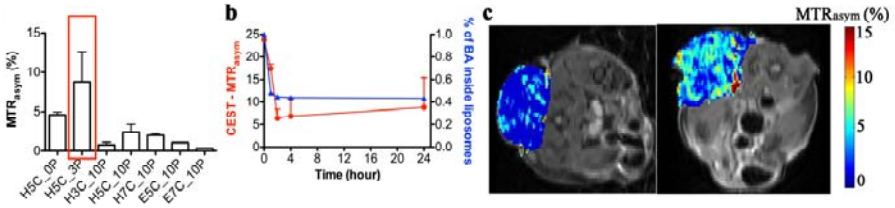

Fig. 2 **a**) CEST contrast at 5 ppm for all formulations after 24 h dialysis, **b**) CEST contrast and quantification of BA inside H5C_3P liposomes at 0, 1, 2, 4 and 24 h. **c**) CEST/T2w overlay images at 5 ppm of a mouse bearing CT26 colon tumor before and after administration of the formulation highlighted in a).