Exploring the inherent CEST MRI signal of anticancer drug gemcitabine

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Target audience: Physicians and other investigators who are interested in imaging drug delivery.

Purpose: To demonstrate that Chemical Exchange Saturation Transfer (CEST) MRI can be used for assessing the uptake and biodistribution of gemcitabine, a chemotherapeutic drug for treating pancreatic cancer without the need for additional MRI contrast agents, providing a direct way to track nanoparticle mediated drug delivery of gemcitabine.

Methods: The CEST properties of gemcitabine were measured on a 9.4 T vertical bore Bruker MRI scanner using a previously reported procedure[1] on phantoms composed of gemcitabine at 1) concentrations ranging from 0.5- 20 mM (pH =7.3), and 2) pH ranging from 6.5 to 8.0 (10 mM). To demonstrate the MRI tracking of drug delivery, gemcitabine encapsulated liposomes (gem-lipo) were prepared using a formulation of DPPC:cholesterol:DPPE-PEG-2000:DPPE-Rhodamine B =55:40:5:0.[2]. The liposome size was measured using dynamic light scattering, and its concentration was determined using colorimetric Stewart assay[2]. The concentration of encapsulated gemcitabine was determined by its absorption at 286 nm after the liposomes were broken using sonication[1]. For in vivo study, 500 µL overnight-dialyzed liposomes (500 mg/kg body weight) were injected in the tail vein of Balb/c mice previously (10 days earlier) implanted with CT26 murine colon tumor cells (subcutaneously, $5x10^6$). In vivo CEST MR images were acquired before and 5 hours after the injection of liposomes on a Biospec11.7 T MRI scanner equipped with a 23 mm mouse brain volume coil. Fat-suppressed CEST MR images were acquired using a continuous wave presaturation pulse (B_1=3.6 µT, 3 sec) with offset incremented from -6 to +6 ppm (0.2 ppm steps); TR/TE=5.0s/5 ms, RARE factor=10. Data were processed using custom-written MATLAB scripts. After correcting B_0 inhomogeneity using the WASSR method, CEST contrast was quantified by $MTR_{asym}=(S^{\Delta\omega} - S^{+\Delta\omega})/ S_0$.

Results and discussion: Figure 1a shows the chemical structure of gemcitabine, which contains both exchangeable amino (blue) and hydroxyl (red) protons, resonating at 2.1 ppm and 1.0 ppm respectively. We first investigated the sensitivity of CEST contrast of gemcitabine and its pH dependency in solution. The results in Figs. 1b and 1c indicate that gemcitabine can be readily detected by CEST MRI, namely MTR_{asym} ~ 0.03 (3.3 Molar signal) at 5 mM drug concentration at the physiological pH. We then tested the use of CEST MRI for tracking gemcitabine encapsulated in liposomes. Use of such nanoparticle drug carriers is a common approach to achieve relative higher local drug concentration than for directly infused drugs. Liposomes are well-established carriers approved for use in the clinic to mediate the delivery of several chemotherapeutic drugs. The results show that after encapsulation of 80 mM gemcitabine (pH = 8.0) in the liposomes, the CEST contrast is well retained in 20 nM liposome solution (Fig 2b). pH 8.0 was chosen because it could generate higher CEST contrast than that prepared at pH 6.5 (Fig 2b). We then injected (i.v.) a solution of 20 nM gemcitabine-loaded liposomes in vivo and imaged 5 hours later. Mice showed marked enhancement in tumor region (Fig 2c, bottom right) compared to that of pre-injection (Fig 2c, top right). The maximal mean CEST contrast enhancement based on manually drawn ROIs of the entire tumor was determined to be 2.6 % in term of MTR_{asym} (n=2) at 3.2 ppm. The MRI findings of drug uptake were confirmed by fluorescence microscopy of PE-Rhodamine.

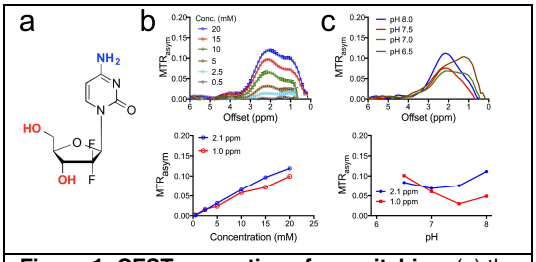

Figure 1. CEST properties of gemcitabine. (**a**) the structure, (**b**) the MTR_{asym} concentration dependence, and (**c**) the MTR_{asym} pH dependence.

Conclusion: In the present study, we demonstrated a direct way to detect an anticancer drug gemcitabine via its inherent CEST MRI contrast, transforming the drug into a theranostic agent. Subsequently, we demonstrated that gemcitabine-loaded liposomes could be directly detected in mouse tumors without the need for any extra imaging agents. Such theranostic MRI has potential to monitor the delivery and biodistribution of the drug and has potential as a predictor of therapeutic outcome. Moreover, because this strategy directly utilizes inherent MRI signal of the drug to be tracked, the need for extra MRI contrast agents is eliminated.

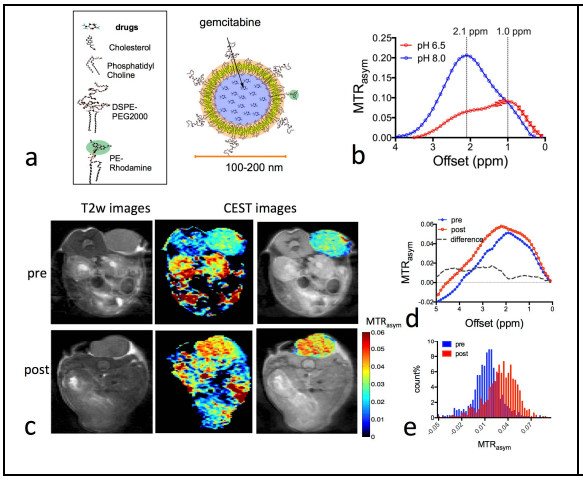

Figure 2. In vivo CEST MRI detection of liposomal gemcitabine. **a:** Illustration of a drug-encapsulated liposome system. **b:** *In vitro* CEST signal of 20 nM gemcitabine liposomes at two different pH values in PBS. **c**: *in vivo* demonstration of detection of liposomal gemcitabine. The T2w MRI and CEST MRI images of a representative mouse before (top) and five hours after injection (bottom). Both whole-slice and overlay CEST images only displaying the contrast within the tumor region are displayed. **d-e**: Quantification of gemcitabine uptake using the mean MTR_{asym} of the whole tumor (**d**) and histogram analysis (**e**) before and after liposome injection.

Reference: (1) Liu, G.; Moake, M.; Har-el, Y. E., *et al. Magn. Reson. Med.* 2012, *67*, 1106-13. (2) Stewart, J. C. *Anal. Biochem.* 1980, *104*, 10-4. **This work is supported by NIH grants R21EB015609, R01EB015032 and R01EB012590.**