Citicoline as a theranostic agent detected by CEST MRI

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Target audience: Physicians interested in imaged-guided drug delivery to treat central neural system (CNS) diseases.

Purpose: 1) To develop cytidine-5'-diphosphocholine (citicoline or CDPC, Fig. 1a), a natural supplement with well-documented neuroprotective effects, as an imaging agent for Chemical Exchange Saturation Transfer (CEST) MRI detection; 2) to construct an image-guided nanoparticle system for the targeted drug delivery of citicoline directly utilizing the CEST MRI signal of citicholine, which can be used to delineate impaired brain regions with a broken blood-brain-barrier (BBB) as well as to treat the injury.

Methods: The CEST properties of CDPC were measured on a 9.4 T vertical bore Bruker MRI scanner using a previously reported procedure[1]. To demonstrate the MRI tracking of drug delivery, CDPC encapsulated liposomes (CDPC-lipo) were prepared using a formulation of DPPC:cholesterol:DPPE-PEG-2000=55:40:5. The liposome size was measured using dynamic light scattering. The concentration of encapsulated citicoline was determined by its absorption at 286 nm after the liposomes were broken using sonication[1]. The CEST contrast of the constructed liposomes (dialyzed at least 5 hours) was confirmed before *in vivo* studies. Transient focal cerebral ischemia in the rat brain was induced by 2-hour middle cerebral artery occlusion (MCAO) with an intraluminal suture as previous reported[2]. After withdrawal of the suture and 60 minutes reperfusion, the extracranial right internal carotid artery (ICA) ipsilateral to the MCAO was cannulated with PE20 Intramedic polyethylene tubing and 1 ml of CDPC-loaded liposomes was infused over a period of 5 min (~50 mg/kg[3]). *In vivo* CEST MR images were acquired before and within the first hour after liposome injection on a Biospec11.7 T MRI scanner equipped with a rat brain surface array RF coil. CEST MR images were acquired using a continuous wave pre-saturation pulse (B_1=3.6 μT, 3 sec) with offsets either of ±1.9, 2.0 and 2.1 ppm, for a dynamic study, or from -4 to +4 ppm (0.2 ppm steps) for the full z spectrum; 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 $MTR_{asym}=(S^{-\Delta\omega} - S^{+\Delta\omega})/ S_0$. The dynamic CEST contrast was quantified by $\Delta MTR_{asym}(t)=MTR_{asym}(t) - MTR_{asym}$ (pre).

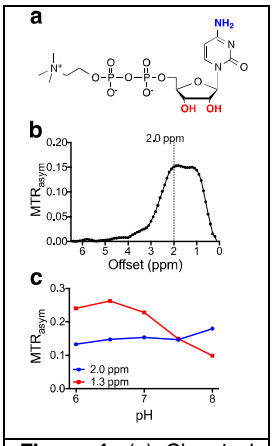

Figure 1. (**a**) Chemical structure and (**b**) CEST properties of citicoline (20 mM pH 7.3); (**c**) the pH dependence of the CEST signal.

Results: As shown in Fig.1b, the CEST MRI signal of CDPC was confirmed by our *in vitro* study, with the 2ppm signal attributed to exchangeable amino protons (blue) and 1ppm signal attributed to hydroxyl (red) protons. The pH dependence study (Fig. 2c) showed that the 2ppm CEST signal is relatively insensitive to pH change, while 1ppm CEST signal is strongly pH dependent and higher at lower pH. We then constructed the CDPC-liposome system for targeted delivery of CDPC. After confirming the CEST signal of CDPC-liposomes, we tested the MRI detectability in rat brain with ischemic stroke (confirmed by DWI MRI, Fig 2a left). The CEST contrast at 1.5 hours post injection showed a marked enhancement in right hemisphere of the rat brain (Fig 2a right). The dynamic CEST MRI data reveal a gradual increase in CEST contrast that reached a maximum at around 50 minutes post-injection (Figs. 2b&c). In contrast, the contralateral brain showed negligible CEST enhancement.

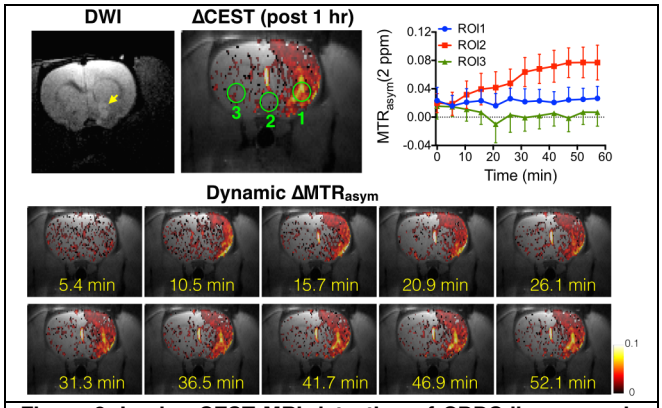

Figure 2. In vivo CEST MRI detection of CDPC-liposomes in ischemic brain. a: DWI and ΔCEST images (1.5 hours post occlusion) of a representative rat **b**: Quantification of the dynamic CEST contrast (ΔMTR_{asym}(2ppm)) to detect CDPC uptake in three regions (shown in figure a); **c**: the ΔMTR_{asym}(2ppm) maps at each time point.

Discussion: Our study reveals that CDPC can be directly detected by CEST MRI, making it possible to use CEST MRI to track CDPC-loaded liposomes without the need of any extra imaging probes. Although CDPC has been used extensively as a natural and safe therapeutics for a variety of CNS diseases, the clinical outcome of CDPC on treating stroke patients however was disappointing[4], which may be due to its low bioavailability if administrated systemically[5] . As evidenced by several recent studies, nanoparticle drug delivery systems including liposomes[5] may greatly boost the bioavailability of CDPC by increasing in the impaired tissues. Our results indicate that CDPC-based drug delivery systems can be inherently image-guided via the CEST MRI signal of CDPC, which can greatly facilitate the clinical translation of CDPC-based drug delivery systems .

Conclusion: In the present study, we demonstrated a direct way to detect the therapeutic agent CDPC via its inherent CEST MRI contrast, making it a theranostic agent. Subsequently, we demonstrated that CDPC-loaded liposomes could be detected in rat brain after stroke directly by CEST MRI signal of CDPC, without the need for any extra imaging agents. Our approach is versatile for developing citicoline-based, (self-) image-guided, nanoparticulate, therapeutics for treating impaired brain.

Reference: (1) Liu, G.; Moake, M.; Har-el, Y. E., *et al. Magn. Reson. Med.* **2012**, *67*, 1106-13. (2) Walczak, P.; Zhang, J.; Gilad, A. A., *et al. Stroke* **2008**, *39*, 1569-74. (3) Ramos-Cabrer, P.; Agulla, J.; Argibay, B., *et al. Int. J. Pharm.* **2011**, *405*, 228-33. (4) Dávalos, A.; Alvarez-Sabín, J.; Castillo, J., *et al. The Lancet* **2012**, *380*, 349-357. (5) Overgaard, K. *J. Stroke Cerebrovasc. Dis.* **2014**, *23*, 1764-9. **This work is supported by NIH grants R21EB015609 and R01EB015032.**