Supplementary Materials

Label-free CEST MRI detection of citicoline-liposome drug delivery in ischemic stroke

Estimation of the r1 and r2 relaxivities of citicoline

To estimate the r1 and r2 relaxivities of citicoline, we prepared citicoline solution at 0, 2, 5, 10 and 20 mM in PBS (pH 7.4). The T1 measurements were measured by using an inversion recovery spin-echo method according to previously published reports. In brief, a 180° hyperbolic secant (sech80) RF inversion pulse (5 ms, bandwidth=15 kHz) was used with 12 different inversion times ranging from 0.5 s to 10 s. A modified RARE sequence was used as imaging readout with 12,000/7.7 msec and RARE factor=16. The resultant MR intensities were fitted as a function of the T_I by $M_{TI}= M0(1-2\exp(T_I/T_1)$ using a home-made Maltab script.

The T2 measurements were made on the same solutions using a Carr-Purcell-Meiboom-Gill (CPMG) preparation period followed by a RARE readout TR/TE= 15,000/40 ms and RARE factor=32). The inter-echo time delay tau was fixed at 10 ms while the number of CPMG preparation modules was varied between 8 and 640 to determine the relaxation rate from the echo time dependence of the signal intensity at constant inter-echo spaces. The resultant MR intensities were fitted as a function of the T_E by $M_{TE}= M0 * \exp(T_E/T_2)$ using a home-made Maltab script.

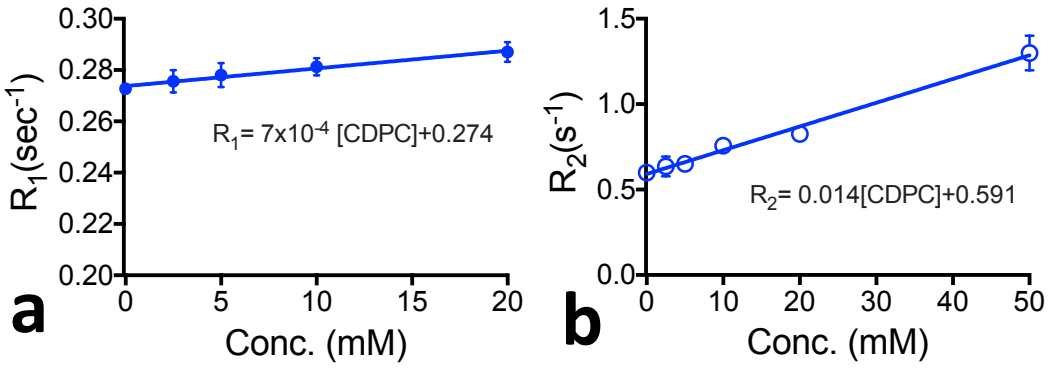

Figure S1. Estimation of r1 and r2 relaxivities of citicoline at pH 7.4 and 37 °C.

Assessment of effects of tissue T_1 and T_2 relaxation times on the CEST MRI detection of citicoline

To assess the effects of tissue T_1 and T_2 relaxation times on the CEST MRI detection, we measured the CEST signal of 10 mM citicoline solution doped with different concentration of Gd-DTPA or $MnCl_2$ as shown in Table S1. The T_1 and T_2 relaxation times of the samples were measured using the MRI methods described in the previous section.

Table S1. Citicoline solutions prepared with different T_1 and T_2

	[Gd-DTPA] (mM)			[$MnCl_2$] (mM)			PBS
	0.01	0.1	0.5	0.01	0.1	0.5	
Measured T1 (s)	2.22	1.70	0.57	2.44	2.29	1.92	3.68
Measured T2 (ms)	1010	970	370	660	440	110	1,460

The CEST signals of these samples were summarized in Figure S2. The shortest T1 (0.57 s, ~6.5 times shorter than that contains no Gd-DTPA) was achieved by 0.5 mM Gd-DTPA, which is shorter than the T_1 in most tissues. The shortest T2 (110 ms, ~ 13 times shorter than that contains no $MnCl_2$) was achieved by 0.5 mM $MnCl_2$, which is similar to T_2 in many tissues. In these cases, the decrease in CEST sensitivity is 70% and 50% respectively, indicating a detectability of several mM can still be obtained for *in vivo* CEST MRI measurement.

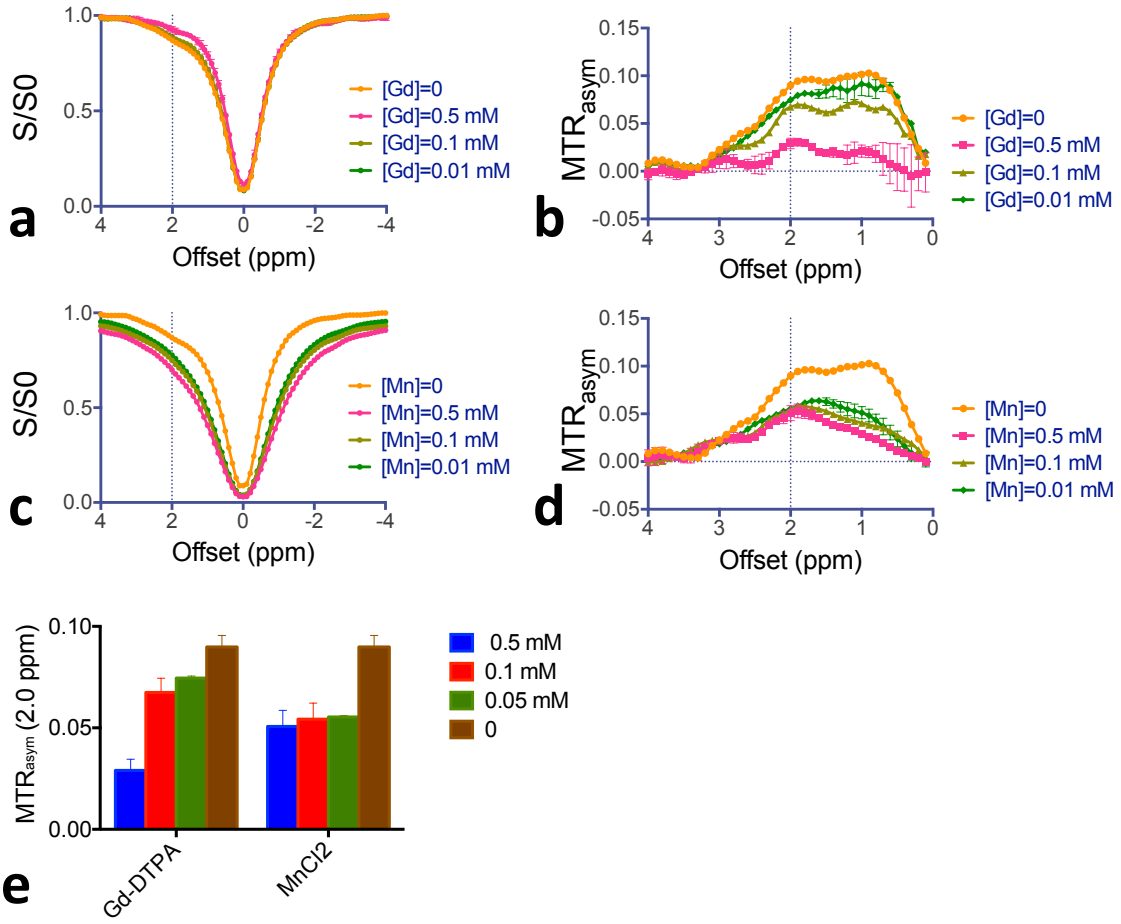

Figure S2. CEST MRI contrast of 10 mM citicoline in solutions with different T1 and T2 relaxation times. (a) The z-spectra and **(b)** MTR_{asym} plots of citicoline solutions doped with 0.01, 0.1 and 0.5 mM Gd-DTPA. **(c)** The z-spectra and **(d)** MTR_{asym} plots of citicoline solutions doped with 0.01, 0.1 and 0.5 mM $MnCl_2$. (e) Bar plots showed that peak CEST values at 2 ppm for all the seven samples measured. All the measurements were carried out at pH 7.4 and 37 ºC.

Estimation of the exchangeable rate constants (k_{ex}) of citicolinee at 2.0 ppm

The exchange rate of exchangeable protons with a frequency of 2 ppm for citicoline at pH=7.4 was measured using the modified *QUantifying Exchange using Saturation Time (QUEST) method* [1]. In brief, the CEST contrast for samples containing 10 mM citicoline (20 mM of amine protons) at pH 7.4 was measured with saturation delays of 1,

2, 3, 4, and 6 sec, using a saturation field strength of 4.7 μT (200 Hz) and a repetition time (TR) set to 10 sec. The calculated MTR_{asym} values were then fit using numerical solutions to the Bloch-McConnell equations with an exchange rate (k_{ex}). The water T_{1w} were experimentally determined to be 3.4 sec, using the inversion recovery method described above. The fixed model parameters were water solute R_{1s}=1 Hz and solute R_{2s}=39 Hz.

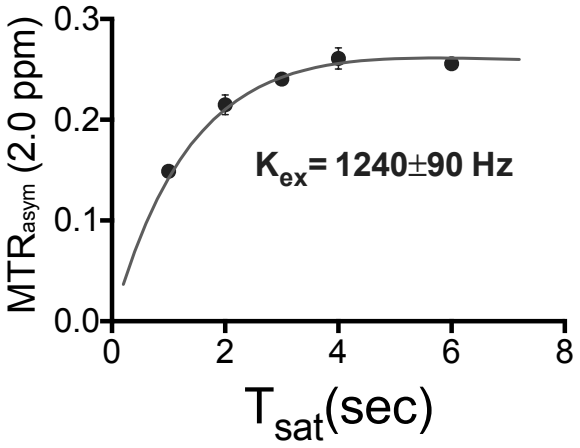

Figure S3. Estimation of the exchange rate of citicoline (2 ppm) at pH=7.4 and 37°C using the QUEST method. Saturation time= 1, 2, 3, 4, and 6 sec, B1= 4.7 μT (200 Hz), TR=10 sec, effective TE=41.4 msec, and RARE factor=16.

Assessment of release rate of citicoline from liposomes in the presence of serum albumin

To determine the release rate of citicoline from liposomes, the retained citicoline in liposomes dialyzed in a sink condition over time were measured according to our previously published procedure[2, 3]. In brief, liposomes were first filtered immediately after formation using a G50 column. One sample was mixed with an equal volume of fetal bovine serum (FBS) to a final volume of 300 μl in a dialysis cassette (3.5

KD MW cut off) [4], and immersed in 50 mL PBS buffer under continuous stirring, which was sufficient to produce a good sink condition. At each time point, i.e., 0, 1, 2, 3, 5, and 24 hours, 150 μL solution from the 50 mL sink was taken to measure the UV absorbance at 286 nm using a UV-Vis spectrophotometer. After the final time point, the sample was suspended in a 10% v/v Triton X-100 solution and sonicated in a water bath at 40 °C for 30 minutes. After centrifugation (21,000 ×g, 10 min), the supernatant were measured for UV absorbance at 286 nm using a UV-Vis spectrophotometer. The result is shown in Figure S4. Compared to that in the absence of serum proteins, the release rate in the presence of serum proteins was much faster. Nonetheless, more than 40% citicoline could be retained in liposomes within the first two hours.

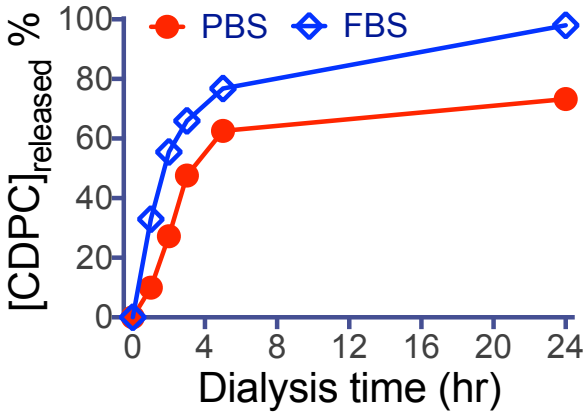

Figure S4. Kinetics of the drug release from liposomes, in the absence and presence of FBS, respectively at different time points studied by UV absorption (OD_{286}).

References:

1. McMahon MT, Gilad AA, Zhou J, Sun PZ, Bulte JW, van Zijl PC. Quantifying exchange rates in chemical exchange saturation transfer agents using the saturation time and saturation power dependencies of the magnetization transfer effect on the magnetic resonance imaging signal (QUEST and QUESP): Ph calibration for poly-L-lysine and a starburst dendrimer. Magn Reson Med. 2006; 55: 836-47.
2. Li Y, Chen H, Xu J, Yadav NN, Chan KW, Luo L, et al. CEST theranostics: label-free MR imaging of anticancer drugs. Oncotarget. 2016; 7: 6369-78.

3. Liu G, Moake M, Har-el YE, Long CM, Chan KW, Cardona A, et al. In vivo multicolor molecular MR imaging using diamagnetic chemical exchange saturation transfer liposomes. Magn Reson Med. 2012; 67: 1106-13.
4. MAGIN RL, NIESMAN MR. Temperature-dependent drug release from large unilamellar liposomes. Cancer Drug Deliv. 1984; 1: 109-17.