Development and application of Dextran CEST MRI agents with improved pH sensitivity

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INTRODUCTION:

The challenges of imaging dextrans using CEST MRI in pathological sites might be complicated by their pH-dependent contrast-enhancement led to our development of second-generation dextran CEST agents that are less sensitive to pH change in the pH range of 6.0-7.4. Here, we demonstrated the effect of chemical modifications on the pH sensitivity of their CEST enhancement and applied relatively pH-insensitive carboxymethyl dextrans to assess the blood-brain barrier disruption associated with neuroinflammation in the EAE mouse model.

METHODS:

Phantom experiments: Dextran (Dex, MW=10 kD, Sigma, # D9260) and carboxymethyl-dextran (CM-Dex, MW=10 kD, Sigma, #86524) were dissolved in PBS buffer (at a glucose monomer concentration of 20 mM) at a range of pHs and imaged using a vertical bore Bruker 11.7 T MRI scanner. CEST MR images were acquired with a RARE sequence and continuous wave pre-saturation pulse (B1=1.2-5.9 μT, 4 sec, TR/TE=6.0s/5 ms, RARE factor=32) for Z-spectra in the range of -5 to +5 ppm (1). The QUESP method was performed to estimate the hydroxyl proton exchange rate at 1.2 ppm (2).

EAE multiple sclerosis mouse model (n=3): C57BL/6 mice (F/6-10w) were injected s.c. with myelin peptide (MOG35-55, 200 μL, 0.5 mg/mL) in incomplete Freund's adjuvant with M. tuberculosis H37Ra (5 mg/mL) and i.p. with 300 ng pertussis toxin on days 0 and 2. Mice were observed daily for paralysis signs. *In vivo* MRI was acquired using a Biospec 11.7 T horizontal MRI scanner and our previously reported protocol (3). CEST images were collected before and after the i.v. injection of 200 μL CM-Dex in saline solution (750 mg/kg b.w.), using the following parameters: B1=1.8 μT, Tsat=3 s, Δω =-3 to +3 ppm with a step size of 0.2 ppm. $MTR_{asym}=(S^{-\Delta\omega} - S^{+\Delta\omega})/S_0$ was computed after B0 correction using the WASSR method. ΔMTR_{asym} (1 ppm) at each time point was calculated by MTR_{asym} (t)-MTR_{asym} (pre).

RESULTS & DISCUSSION:

Chemically modified dextrans show distinctive CEST contrast due to modulated OH exchange rates. We first compared the CEST signals of unmodified Dex, CM (negatively charged) modified Dex, and spermine (positively charged) modified Dex. Our results reveal the addition of CM led to an enhanced CEST, while spermine strongly suppressed the CEST signal at pH 7.4. We estimated the exchange rate of CM-Dex is 2.3 kHz at pH 7.4, which is much slower than that of unmodified Dex (4.6 kHz). As a result of CM modification, CM-Dex exhibits only a weak dependency on pH in the pH of 6 to 7.4 (**Fig 1**), which is highly desirable for *in vivo* applications.

CM-Dex-enhanced CEST MRI revealed contrast in brain regions prone to BBB impairment. Regions of enhancement were heterogeneous (**Fig 2**) and varied among EAE mice. Although CM-Dex (10 kDa) is larger than Gd-DOTA (559 Da), the area of enhancement was slightly larger in CM-Dex-enhanced CEST maps than that by Gd, suggesting that other particle properties other than molecular weight (MW) might also contribute to tissue uptake when BBB is compromised. The exact confounding factors and their contribution are still under investigation.

CONCLUSION:

We developed a second-generation CEST agent, CM-Dex, that exhibits improved pH dependency in the 6.0-7.4 pH range. We demonstrated its utility for evaluating BBB integrity in the EAE mouse model. Studies to evaluate the accuracy of CEST MRI quantification of CM-Dex at *in vivo* compartments of different pH are ongoing.

REFERENCES:

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3. Han Z, *et al.* NMR Biomed 2022;35(3):e4649.

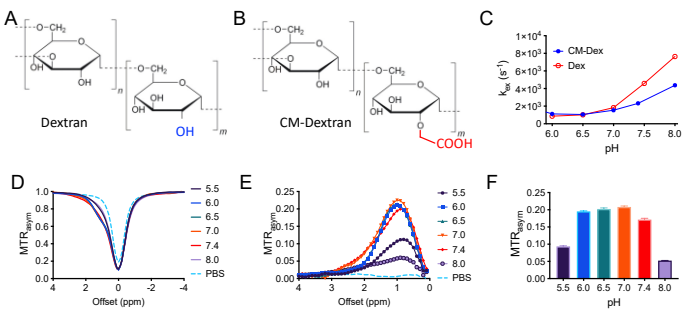

Figure 1. (A,B) Chemical structures of dextran and carboxymethyl-dextran, **(C)** Exchange rates of OH in Dex and CM-Dex, **(D)** pH-dependence of 20 mM CM-Dex at 1.2 ppm characterized by Z-spectra, **(E)** MTR_{asym} plots, **(F)** pH dependency of MTR_{asym} at 1.2 ppm.

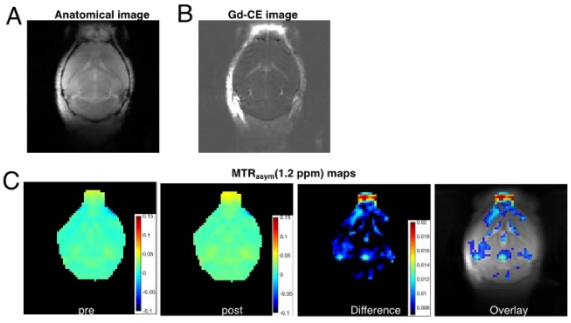

Figure 2: Dex-enhanced CEST MRI of a mouse (score 1.5) at 20 min post-CM-Dex injection. **(A)** T2w image, **(B)** Gd-enhanced image**,** and **(C)** CEST MRI**.** From left to right: pre- and post- CEST images, and contrast enhancement maps**.**