MRI detection of bacterial infection through endogenous CEST contrast

Guanshu Liu[1,2], Yuan Qiao[3], Chetan Bettegowda[3], Verena Staedtke[3], Kannie W.Y. Chan[2,4], Renyuan Bai[3], Gregory Riggins[3], Kenneth W Kinzler[3], Jeff W.M. Bulte[2,4], Michael T McMahon[1,2], Assaf A Gilad[2,4], Bert Vogelstein[3], Shibin Zhou[3], and Peter C.M. van Zijl[1,2]

[1]F.M. Kirby Research Center for Functional Brain Imaging, Kennedy Krieger Institute, Baltimore, MD, United States, [2]Department of Radiology, Johns Hopkins University School of Medicine, Baltimore, MD, United States, [3]Ludwig Center, Howard Hughes Medical Institute and Sidney Kimmel Cancer Center, Johns Hopkins University School of Medicine, Baltimore, MD, United States, [4]Cellular Imaging Section, Institute for Cell Engineering, Johns Hopkins University School of Medicine, Baltimore, MD, United States

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

Purpose: To develop a non-invasive MRI method for determining the sites and extent of bacterial infection using CEST contrast and apply it to monitor anti-cancer therapy with tumor-homing bacteria.

Methods: The bacterium of *C. novyi-NT* (*C. novyi* without toxin gene) was cultured under anaerobic conditions.[1] DH5alpha *E. coli* was purchased from Invitrogen and culture in LB medium. Colorectal HCT116 cells ($5x10^6$) were injected subcutaneously into the flank of athymic nude mouse to form tumors. Xenografts were allowed to grow for ~14 days to reach critical size (> 350 mm^3) with highly hypoxic cores. To monitor the germination *in vitro*, spores were incubated in 2% agar gel and culture broth at a final concentrations of 0 to $4x10^6$ /ml in 5 mm NMR tubes inside the MRI scanner at a temperature of 37 °C for a series of intermittent MRI acquisitions over 6 hours. For monitoring *C. novyi-NT* treatment *in vivo*, MRI acquisition was conducted before and 24h after tail vein injection of spore solution ($3x10^8$ spores in 200µl PBS). The time course of 24h post-injection allows bacterial germination and the occurrence of an immune response. For detecting *E. Coli* infection, the MRI acquisition was conducted before and immediately after intratumorally injecting 50 µL *E. coli* (10^7cells/ml in saline) solution. *In vivo* CEST acquisitions were conducted as described previously[2] on a Bruker BioSpec 11.7T imaging system. A modified RARE (TR=5.0 sec, effective TE = 43.2ms, RARE factor =8, slice thickness=1 mm, matrix size=128x64 and NA=2) including a magnetization transfer (MT) module (3 sec CW pulse, B1= 3.6 µT). The frequency sweeping range was -5 ppm to 5ppm (step=0.4 ppm). WASSR method was used to correct B_0 inhomogeneities. CEST was quantified by $MTR_{asym}=(S^{-\Delta\omega} - S^{+\Delta\omega})/ S_0$.

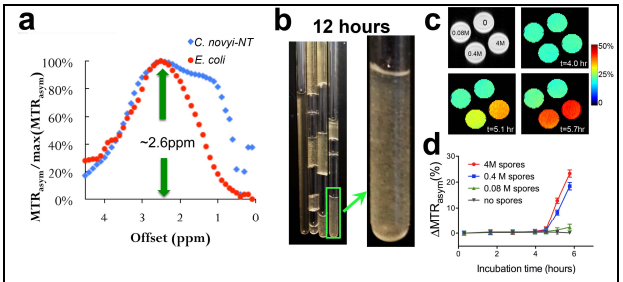

Fig. 1. **CEST MRI detection of bacteria in vitro. a**) The endogenous CEST contrast of *C. novyi-NT* and *E. coli* as shown by their normalized MTR_{asym} plots (by its maximal value) of bacterial PBS solutions. **b)** appearance of tubes with spores after 12 hours of 'incubation' inside the MRI scanner at 37 °C; **c)** T2w images and MTR_{asym} maps at 2.6 ppm of *C. novyi-NT* spores at 4.0, 5.1 an 5.7 hours after intra-scanner incubation (37°C), and **d)** Time course of CEST contrast for four samples, displayed as $\Delta MTR_{asym} = MTR_{asym}(t) - MTR_{asym}(0)$, where $MTR_{asym}(t)$ is the mean CEST contrast at 2.6 ppm at time t after incubation, and $MTR_{asym}(0)$ is that measured at the start of incubation.

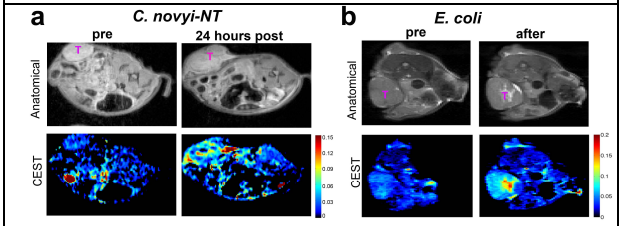

Fig. 2. **CEST MRI detection of bacterial infection in tumors. a**) T2w (anatomical) and CEST contrast (MTR_{asym} maps at 2.6 ppm) of the tumor of a mouse before and 24 hrs after intravenous injection of *C. novyi-NT* spores; **b**) T2w and CEST contrast (MTR_{asym} maps at 2.6 ppm) of *E. coli* infection in a mouse tumor before and immediately after intratumoral injection of *E. coli* solution.

Results: *In vitro* results showed that solution containing living bacteria exhibited a broad CEST signal with the maximum CEST contrast at 2.6 ppm (Fig.1a). To test if this technology can be used for other bacteria, we also investigated *E. coli*. When examined in test tubes, *E. coli* exhibited a similar frequency-dependent pattern (Fig.1a) but a narrower curve compared to *C. novyi-NT*. The ability to detect bacterial germination using CEST was demonstrated on a phantom composed of three tubes containing spores at different concentrations and one tube containing only culture medium (Fig. 1b,c). After incubation at 37 ºC for several hours, *C. novyi-NT* spores spontaneously germinated. CEST MRI clearly detected the bacterial germination and proliferation that occurred between 4 and 6 hrs (Figs. 1c,d).

When applied to bacteriolytic therapy, CEST contrast in the tumor was compared before and 24 hours after intravenous injection of *C. novyi-NT* spores. The CEST signal at 2.6 ppm was dramatically elevated inside the tumor (Fig. 2a), indicating bacterial infection in this area, which was confirmed by histology. Quantitative analysis for four mice showed a significant increase of mean MTR_{asym} (2.6ppm) from 2% to 4.9% ($P< 0.01$, Student t test, two tails, paired). Using the CEST contrast offset of 2.6ppm, we also successfully detected the directly injected *E. coli* in tumors (Fig.1b).

Discussion: The data in this paper demonstrate that MRI can be used for monitoring the germination and proliferation of bacteria directly through their endogenous bulk CEST contrast. This is likely due to exchangeable protons from a variety of bacteria-specific molecules such as cellular carbohydrates, peptides and proteins and several metabolites.[3,4] The immediate significance of this method lies in its potential to image infection as demonstrated by the successful monitoring of *C. novyi-NT*-based tumor therapy through the early detection of germination.

Conclusion: We used the endogenous CEST signal of bacteria at 2.6 ppm to detect infection of colorectal tumor with both the gram positive bacteria *C. novyi-NT* and gram negative *E. coli*, showing the potential for non-invasive detection of bacterial infection in vivo without the need for contrast agents.

Reference: (1)Dang, L. H., *et al. Cancer Biol. Ther.* **2004**, *3*, 326-37.(2)Liu, G., *et al. Magn. Reson. Med.* **2012**, *67*, 1106-1113.(3)van Zijl, P. C., *et al. Proc. Natl. Acad. Sci. U. S. A.* **2007**, *104*, 4359-64.(4)Zhou, J., *et al. Nat. Med.* **2003**, *9*, 1085-90. This work is supported by NIH grants R01EB015032, R01EB012590, R21EB015609, R21EB008769, R21EB005252, and P50 CA062924.