INTRODUCTION

Anaerobic bacteria have been known for more than 50 years for their ability to exclusively proliferate in hypoxic solid malignancies, resulting in tumor destruction. This approach, named bacteriolytic therapy, has found renewed interest through the availability of genetic engineering of various bacteria. For example, *Clostridium novyi-NT*, the bacterium explored here is currently being tested in a FDA approved phase I clinical trials of colorectal cancer. In experimental models, after a single intravenous administration of *C. novyi-NT* spores, the bacterium generates a robust and predictable response consisting of germination and proliferation within the tumor in 12-24 hours, followed by an immune responses to eradicate tumor cells in the well oxygenated regions. A non-invasive imaging approach that could detect this treatment response would be of immense value for translating bacteriolytic therapy into the clinic. We hypothesized that bacteria should be detectable through their high protein content using Chemical Exchange Saturation Transfer (CEST) MRI, which has been used to detect intracellular mobile proteins using the so-called amide proton transfer (APT) signals at 3.5 ppm from water protons. These signals are higher in tumor cells, which have been extensively studied for their CEST signal. To test whether we can detect bacteria through their proteins, we investigated the CEST properties of *C. novyi-NT* in vitro, followed by in vivo exam of whether tumor infection could be measured by CEST MRI.

METHODS AND MATERIALS

The bacterium of *C. novyi-NT* (*C. novyi* genetically engineered to remove the major systemic toxin gene) was anaerobically cultured. For the *in vitro* study, bacteria were re-suspended in 10 mM PBS containing 2% oxyrase for an anaerobic environment. The concentration of bacteria was estimated by their O.D. 600 nm and then adjusted to three different concentrations (3x10^6, 6x10^6 and 3x10^7 cells/ml). Samples of 2% oxyrase in PBS and spore solution (4x10^8 spores/ml) were also prepared as controls. In *in vitro* CEST MRI was conducted as described on a 9.4T Bruker imager. A modified RARE (TR=6.0 sec, effective TE = 43.2ms, RARE factor =16, slice thickness=0.7mm, matrix size=128x64, resolution= 0.10x0.15mm², and NA=2) including a magnetization transfer (MT) module (3sec continuous pulse, B1= 3.6 μT) was used to acquire CEST weighted images with saturation sweep from -5ppm to 5ppm (step=0.4 ppm) with respect to water resonance (0ppm). The B0 inhomogeneity was corrected using the WASSR method. CEST was quantified using MTR asym, as defined by (S asym - S 0)/S 0. 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 mm³) with highly hypoxic cores. First, an MRI acquisition was conducted before injection to assess the background CEST signal. Subsequently, each animal received tail vein injection of spore solution (3x10^8 spores in 200μl PBS). A second CEST MRI was conducted ~ 24h hours after injection to allow bacterial germination and the occurrence of an immune response. In *in vivo*, the same CEST acquisitions were used as in *in vitro*, except for TR= 5 sec and RARE factor =8.

RESULTS AND DISCUSSION

*In vitro* results (Fig. 1) clearly showed pronounced CEST MTR asym signal at 2.6ppm and 1ppm after the background subtraction of CEST signal of medium. A good linear correlation between apparent MTR asym and concentration was found (Fig. 1b), indicating that the CEST signal at 2.6ppm can potentially be used to quantify bacterial infection. In *in vitro* results show 5% CEST contrast for a density of 100 cells/voxel (0.0145 mm^3/ voxel), implying the CEST method is feasible for *in vivo* studies.

We then injected *C. novyi-NT* spores intravenously into tumor bearing mice and observed the change in CEST signal before and 24 hours after injection, by which time the bacteria had germinated in the hypoxic regions of the tumor and the immune responses had already been triggered as evidenced by gram staining (Fig. 2d). As shown in Fig.2, the CEST signal at 2.6 ppm was dramatically elevated inside the tumor, indicating bacterial germination in these areas. The CEST signal in the tumor was very heterogeneous and we therefore used histogram analysis of the entire tumor to analyze it. When fitting the histogram of the CEST signal to a Gaussian distribution the contrast at 2.6 ppm (bacterial infection) was found increased from 2.0 ± 2.0% to 5.3± 3.3%. Based on the *in vitro* calibration, we estimated an average cell density of approximately 3.5 million cells/ml in the tumor or approximately 1.2x10^8 bacteria in the 1mm thick image plane.

CONCLUSION

We used the endogenous CEST signal of bacteria at 2.6 ppm to detect infection of colorectal tumor xenografts with the therapeutic bacterium *C. novyi-NT*, showing potential for non-invasive monitoring of bacteriolytic treatment in *in vivo* without the need for contrast agents.

REFERENCE