Published in final edited form as:

Magn Reson Med. 2013 December ; 70(6): . doi:10.1002/mrm.24955.

Non-invasive imaging of infection after treatment with tumor-homing bacteria using chemical exchange saturation transfer (CEST) MRI

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Abstract

Purpose—To develop a non-invasive MRI method for determining the germination and infection of tumor-homing bacteria in bacteriolytic cancer therapy using endogenous CEST contrast.

Methods—The CEST parameters of the anaerobic gram-positive bacterium *Clostridium novyi-NT* (*C. novyi-NT*) were first characterized *in vitro*, then used to detect *C. novyi-NT* germination and infection in subcutaneous CT26 colorectal tumor-bearing mice (n=6) after injection of 300 million bacterial spores. Lipopolysaccharide (LPS) injected mice were used to exclude that the changes of CEST MRI were due to inflammation.

Results—CEST contrast was observed over a broad frequency range for bacterial suspensions *in vitro*, with the maximum contrast around 2.6 ppm from the water resonance. No signal could be detected for bacterial spores, demonstrating the specificity for germination. *In vivo*, a significant elevation of CEST contrast was identified in *C. novyi-NT* infected tumors as compared to those before bacterial germination and infection (p<0.05, n=6). No significant change was observed in tumors with LPS-induced sterile inflammation (p> 0.05, n=4).

Conclusions—Endogenous bacterial CEST contrast (bacCEST) can be used to monitor the germination and proliferation of the therapeutic bacterium *C. novyi-NT* without a need for exogenous cell labeling probes.

INTRODUCTION

It has been shown that certain bacteria, when injected into mice, can selectively infect experimental tumors (1–3). This approach to therapy has a long and venerable history, as unintended bacterial infection has been observed to be associated with tumor regression for more than 300 years, and purposeful injection of bacteria into tumors has been attempted in patients for over a hundred years (4,5). Recent studies showed that certain bacteria are capable of spontaneously homing to and selectively growing in the hypoxic core of tumors

(6,7). For anaerobic bacteria, this selective growth is due to the fact that the only environment sufficiently hypoxic for bacterial germination is within tumors, although other factors may also play a role (2,3,7). This unique cancer-tropism makes such bacteria ideal carriers for targeted delivery of anti-cancer payloads, such as genes encoding prodrug-activating enzymes. Moreover, bacterial growth can be easily terminated with antibiotics, making the side effects of these therapeutics controllable (4,8–14). As a result, numerous bacterial species, including *Salmonella* (15–17), *Clostridium* (11,18,19), *Bifidobacterium* (10), and *Escherichia* (20), have been reported to have therapeutic utility in experimental tumor systems (1,2,11,12,17,21,22). These approaches have been collectively termed “bacteriolytic therapies”.

We have genetically modified *C. novyi* to create a new strain, called *C. novyi-NT*, that is devoid of the major systemic toxin of the parental strain. We have shown that these bacteria are exquisitely sensitive to oxygen, can only germinate within tumors, and can be used either alone or in combination with other therapeutic agents to induce regression and cures in many different tumors in mice and rabbits (3,19,21,22). *C. novyi-NT* also triggers a robust immune response that is partly responsible for its efficacy (11,21). It is currently in a Phase I clinical trial in the United States (NCT01118819).

Non-invasive imaging of the distribution, accumulation, proliferation and clearance of injected therapeutic bacteria should be extremely useful for the evaluation of bacteriolytic therapies. While a number of approaches using optical imaging (6,23–27) and nuclear imaging (28,29) have been reported, a clinically translatable MRI method would be especially attractive in view of its superb soft tissue contrast, high spatial resolution, and absence of ionizing radiation. Several anatomical and functional MRI methods have been used to visualize infectious areas and abscesses, including T1-weighted MRI (hypointensity), T2-weighted MR (hyperintensity), gadolinium based dynamic contrast enhanced MRI (peri-lesional contrast enhancement), and diffusion weighted MRI (low apparent diffusion coefficients) (30–32), but these approaches often have limited specificity. MR spectroscopy (MRS) can identify bacteria-specific metabolic markers in the proton spectrum, for instance amino acids (0.9 ppm), lipids (0.9 and 1.2 ppm), lactate (1.4 ppm), acetate (1.9 ppm) and succinate (2.4 ppm) (33) and have been used to distinguish bacterial abscesses from malignant gliomas and tuberculomas (34–36). However, while quite specific, the application of MRS in the clinic is often limited by its inherently low spatial and temporal resolution.

Chemical Exchange Saturation Transfer (CEST) is a relatively new MRI contrast mechanism (37,38) that has been developed extensively in the last decade (39–42). CEST utilizes the chemical exchange between an exchangeable proton and its surrounding water protons to transfer the selectively modulated NMR signal from exchangeable protons to water and consequently to produce MRI contrast. Because the water protons have much higher concentration (~110 M) as compared to the targeted exchangeable protons (typically on the order of mM) and because the CEST effect produces sensitivity enhancement proportional to the exchange rate, the MRI detectability of low concentration molecular targets using a CEST approach can be boosted by several orders of magnitude, providing a new way to realize MR molecular imaging with water sensitivity. Depending on the exchange rate, enhancements by factors of 100–1000 are routinely achieved (see several review papers: refs. 39–41). CEST contrast raised from endogenous molecules, or endogenous CEST contrast, has been explored for the detection of tumor cells (43,44), or pH, metabolic processes and metabolites (45–49). In the current study, we evaluated the possibility of diamagnetic Chemical Exchange Saturation Transfer (CEST) MRI to detect bacterial germination (Figure 1a). Although the same type of exchangeable protons, including those from amine, hydroxyl, and amide groups, are also present in mammalian

cells, we postulated that bacterial cells could be detected through their differential endogenous CEST contrast without the need for additional MRI contrast agents because they have 1) a different chemical composition (50), 2) a different metabolism and metabolic rate (33,34,36,51), and 3) a much smaller cell size or larger surface to volume ratio, which allows more saturated intracellular protons to be exchanged to outside via water transport, as revealed by several recent studies on how particle size affects the CEST contrast of liposome-entrapped agents (52,53). To test this hypothesis, we evaluated the CEST spectra of *C. novyi-NT* *in vitro* and then applied CEST MRI at the optimal frequency offset for this bacterium to assess signal changes in subcutaneous tumors in mice following systemic administration of spores.

METHODS

Cell culture

Human HCT116 colorectal cancer cells and murine CT-26 colon carcinoma cells were purchased from ATCC and grown as monolayers in McCoy 5A medium (Life Technologies, Rockville, MD) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. Vegetative *C. novyi-NT* was anaerobically cultured in Reinforced Clostridial Medium (RCM) (Difco). *C. novyi-NT* spores were prepared following procedures described previously (18). The concentration of bacterial spores or vegetative bacteria was determined via optical absorption at 600 nm.

Animals

Animal experiments were performed in accordance with protocols approved by Johns Hopkins Institutional Animal Care and Use Committee. To form tumors, five million CT-26 cells were subcutaneously injected into the right flank of BALB/c mice (female, six to eight week-old, from Harlan Laboratories, Frederick, MD). Tumor volume was estimated as $\text{length} \times \text{width}^2 \times 0.5$. Syngeneic tumors were allowed to grow for ~14 days to reach a size ($>350 \text{ mm}^3$) suitable for robust *C. novyi-NT* germination. Animals (n=6) received a tail vein injection of *C. novyi-NT* spores (3×10^8 spores in 200 μL PBS) and were imaged 12 hours later to allow bacterial germination (11,21,22).

To investigate the CEST-MRI signal of lipopolysaccharide (LPS) induced sterile inflammation, animals (n=4) received intratumoral injections of LPS at a dose of 1 mg/kg body weight in a volume of 50 μL and imaging was performed 24 hours later, when the inflammatory response was noticeable (26,54).

MRI

***In vitro* CEST MR imaging**—Prior to *in vitro* MRI, cultured *C. novyi-NT* were harvested and re-suspended in 10 mL PBS or culture medium containing 2% Oxyrase (Oxyrase Inc.), which was used to maintain an anaerobic environment (55). To compare the CEST contrast of bacteria and spores, 6×10^6 cells/mL *C. novyi-NT* and 4×10^7 spores/mL were re-suspended in PBS containing 2% Oxyrase and transferred into glass capillaries (inner diameter=1.1–1.2 mm) that were inserted into a home-made sample holder (56). To determine the concentration dependency of CEST contrast, *C. novyi-NT* was re-suspended in culture medium containing 2% Oxyrase at final concentrations of 13, 6.5, 3.0, and 1.3×10^7 cells/mL. To investigate the pH influence on the CEST contrast of bacteria, *C. novyi-NT* was re-suspended at a final concentration of 6×10^7 cells/mL in culture medium with the pH adjusted to 5.5, 6.0, 6.5, 7.0 and 7.5 (a pH range that won't affect the integrity of bacterial cells (57,58)). To monitor the germination of spores *in vitro*, spores were re-suspended in culture broth mixed with 2% pre-heated agar gel at final concentrations of 4, 0.4 and 0.08×10^6 spores/mL, and transferred to 5 mm NMR tubes to allow gelation at room temperature.

Subsequently, these samples were transferred to a vertical bore MRI scanner for a series of intermittent MRI acquisitions over a period of 6 hours with probe temperature maintained at 37 °C.

All *in vitro* MRI was performed on a vertical bore 9.4 T Bruker Avance system equipped with a 15 mm volume RF coil. A modified RARE (TR/effective TE = 6000/43.2 ms, RARE factor=16, slice thickness=0.7 mm, FOV=14×14 mm², matrix size=128 × 64, resolution=0.11 × 0.22 mm², and 2 averages) including a magnetization transfer (MT) module with saturation time (t_{sat}) of 3 s and radiofrequency field strength (B1) of 3.6 μ T was used to acquire CEST weighted images from -6 to 6 ppm (0.2 ppm steps) around the water resonance (0 ppm), constituting a CEST spectrum (also called Z-spectrum) (56). With an acquisition time of 24 s/image, each Z-spectral acquisition took approximately 24 minutes. The absolute water resonance frequency shift was imaged using a modified Water Saturation Shift Reference (WASSR) method (56,59) with the same parameters as used for CEST imaging except that TR=1.5 s, t_{sat} =500 ms, B1=0.5 μ T, and sweep range= -1 to 1 ppm (0.1 ppm steps).

***In vivo* CEST imaging**—*In vivo* MRI was conducted on a horizontal bore 11.7 T Bruker Biospec system using a 23 mm quadrature volume RF coil. First, an acquisition was conducted before the injection of spores or LPS to assess the background CEST signal. A second CEST-MRI scan was conducted about 12 hours after the injection of spores or about 24 hours after the injection of LPS. The protocol used for *in vivo* CEST acquisitions was the same as *in vitro*, except for TR of 5 s and a RARE factor of 8. With an acquisition time of 40 s/image, each Z-spectral acquisition took approximately 30 minutes.

Data processing—Data processing was performed using custom-written scripts in MATLAB. Z-spectra were calculated from the mean ROI for each sample after B0 correction. CEST signals were quantified as described previously (60) using the asymmetry in the magnetization transfer ratio with respect to the corrected water frequency at 0 ppm (MTR_{asym}) at particular offsets of interest (i.e. $\Delta\omega = +2.6$ ppm) using $\text{MTR}_{\text{asym}} = (S_{-\Delta\omega} - S_{+\Delta\omega})/S_0$, where $S_{-\Delta\omega}$ and $S_{+\Delta\omega}$ is the water signal in the presence of a saturation pulse at offsets $\pm\Delta\omega$ with respect to the water resonance and S_0 is water signal in the absence of a saturation pulse.

Histology

Hematoxylin/eosin (H&E) and Gram stainings were performed as described previously (11,22).

Statistics

Values were calculated as mean $\pm$ s.d. Two tailed non-equal variation student's t-tests were used to determine differences ($P < 0.05$).

RESULTS

Endogenous CEST contrast enables direct visualization of bacteria using MRI

We first characterized the endogenous CEST contrast of *C. novyi-NT* *in vitro* and compared it with that of spores. As shown in Figures 1b and c, a solution containing the vegetative form of *C. novyi-NT* (6×10^6 cells/mL in PBS) exhibited strong CEST contrast (defined as mean $\text{MTR}_{\text{asym}} > 3$ times the standard deviation (SD) over the pixels in the ROI, which was approximately 0.017 here) over a broad chemical shift range from 0.5 ppm to 4 ppm, while the contrast for spores (4×10^7 cells/mL in PBS) produced no detectable CEST signal (defined as $\text{MTR}_{\text{asym}} < \text{SD}$). The broad CEST signal indicates that it is likely that more than

one functional group contributes to the bacterial CEST contrast. The highest CEST effect was observed around a saturation offset of 2.6 ppm, which was therefore used for CEST imaging in subsequent studies. The presence of 2% Oxyrase, which was used to maintain an anaerobic environment (55) did not affect CEST detection of bacteria as confirmed by the fact that CEST contrast was negligible in a solution of Oxyrase in PBS (supporting information).

We subsequently investigated the concentration dependence of bacterial CEST contrast by re-suspending *C. novyi-NT* at multiple cell densities in fresh cell culture broth composed of 10% fetal bovine serum (FBS) and a variety of nutritional supplements with a measured pH of 6.5. This pH represents the lowest value that can be encountered *in vivo*. As expected, the presence of serum proteins and other nutrition supplements resulted in a notable CEST background contrast (supporting information). To remove such background signal, the net bacterial CEST contrast of each sample was calculated by subtracting the CEST signal of culture medium without bacteria from that of medium with bacteria. Even at the lowest concentration studied (1.3×10^7 cells/mL in medium), the *C. novyi-NT* cells still exhibit detectable net CEST contrast (0.020 ± 0.003 change in MTR_{asym}). For our MRI spatial resolution of 0.0145 mm³/voxel, this cell density corresponds to approximately 189 cells/voxel, which implies that the proposed CEST method should in theory be capable of detecting bacteria at concentrations found *in vivo*. As shown in Figure 1d, the CEST contrast of *C. novyi-NT* was found to correlate well with cell density.

As pH can significantly affect the exchange rate through base or acid catalysis of the proton exchange process, we determined the effect of pH effect on the CEST contrast. Somewhat surprisingly, the net CEST contrast of *C. novyi-NT* in culture broth did not change much when varying the extracellular pH from 5.5 to 7.5. This phenomenon may be due to the intra-cellular pH of bacteria remaining unchanged through homeostasis, but needs further investigation. Independent of the mechanism, this result is important because it indicates that CEST MRI assessment of bacteria would not be significantly affected by the extracellular pH within tumors, which can vary considerably.

Monitoring the germination of *C. novyi-NT* *in vitro* using CEST MRI

To investigate the ability to detect bacterial germination using CEST, we prepared a phantom composed of three tubes containing spore suspensions at different concentrations and one tube containing only culture medium (Figure 2a). To prevent possible aggregation and precipitation, we used 2% agar gel to fix the spores and vegetative bacterial cells (after germination). After incubation in the MRI scanner at 37 °C for several hours, spores spontaneously germinated. For longer incubation periods (12 hours), the germinated bacteria produced enough hydrogen gas to break apart the agarose gels in which they were embedded (Figure 2a). Before this occurred (e.g., <6 hours) CEST MRI had clearly detected bacterial proliferation (Figures 2b and c). Higher spore inoculation resulted in an increase in CEST contrast (Figure 2c). However, this increase is not proportional to concentration, which may be due to the back exchange of saturated signal at high concentration.

Monitoring the germination of *C. novyi-NT* in subcutaneous colon tumors

CT-26 solid tumors have been shown to harbor sufficiently hypoxic regions to support *C. novyi-NT* germination within the first 24 hours after intravenous injection of spores (21,22,61). Using this model, we monitored the CEST signal before and 12 hours after systemic spore administration. The anatomical MR images, as shown in Figure 3a, revealed an increase in the hyper-intense T2-weighted (T2w) MRI signal around the tumor, which may be due to edema and a local inflammatory response. However, no particular change in contrast intensity was seen. The CEST signal (MTR_{asym} at 2.6 ppm), on the other hand, was

found to be dramatically increased, in a good agreement with bacterial infiltration in these areas (Figure 3a).

Conventional ROI based analysis of the entire tumor region (Figure 3c) clearly showed the changes associated with bacterial infiltration. The mean $MTR_{asym}(2.6 \text{ ppm})$ showed a significant increase from 0.047 ± 0.015 in mice prior to treatment to 0.065 ± 0.019 in post *C. novyi-NT*-treated mice ($P < 0.05$, Student t test, two-tailed paired). Based on the *in vitro* calibration (Figure 1d), we estimated an average cell density of approximately 1×10^7 cells/mL in the tumor. However, analysis of the entire tumor as one entity is not optimal because *C. novyi-NT* are primarily localized to focal areas of hypoxia rather than distributed randomly throughout (Figure 3b and refs. 11,19,21 and 22). To overcome this limitation, we also performed histogram analysis of the CEST signal of all voxels that were classified as intra-tumoral based on the corresponding T2w image (Figure 3d). A noticeable group of voxels at 12 hours after injection was clearly shifted to higher CEST signal as compared to before injection, indicating that a good portion of tumor voxels exhibited increased CEST contrast.

LPS-induced inflammation does not cause an increase in CEST contrast

To investigate whether bacterial CEST contrast can be influenced by the inflammation that always accompanies bacterial infection, we also measured CEST contrast after injecting LPS to induce sterile inflammation within the tumors (Figure 4). Twenty-four hours post-injection, significant hemorrhage and necrosis were found in the tumor center, while neutrophil infiltration was found at the tumor periphery (Figure 4b), as expected from the sterile inflammatory response typically induced by LPS (62). While it is difficult to control the extent of inflammation induced by LPS to be exactly the same as that by bacteria, the comparison of ex vivo histopathology of LPS-induced inflammation (Figure 4b) and that of bacteria-induced inflammation (Figure 3b, as well as Figure 4 in ref. 11 and Figure 1 in ref. 22) reveals a similar pattern. In contrast to the tumors treated with *C. novyi-NT* spores (Figure 4), the CEST contrast of tumors undergoing sterile inflammation was not increased noticeably in comparison to untreated tumors (Figure 4a right panel). Quantitative ROI analysis showed that the mean $MTR_{asym}(2.6 \text{ ppm})$ of the whole tumor ROI was not significantly altered (Figures 4c, e), which was confirmed by histogram analysis (Figure 4d).

DISCUSSION

The results indicate that CEST MRI can be used for monitoring the germination and proliferation of bacteria without the need for genetic modification or the use of exogenous imaging probes. This noninvasive approach has potential to be quickly translated to the clinic as CEST-MRI has already been demonstrated to be achievable at field strengths down to 3 Tesla (63,64). Here, as a proof-of-concept, we were able to show that *C. novyi-NT* can be directly detected through its intrinsic CEST contrast over a frequency range from 0.5 ppm to 4 ppm, with a maximum at 2.6 ppm. This contrast is likely to be due to exchangeable protons from a variety of bacteria-specific macromolecules such as cellular carbohydrates (49,65,66), peptides (67,68) and proteins (45), or metabolites (38,49,60,69). We also found that the spore form of *C. novyi-NT* did not show significant CEST contrast, which can be attributed to the remarkably low water (70), little and no metabolism (71), extremely low water permeability (72) and a tightly assembled outermost, known as the coat, consisting of highly cross-linked proteins (73). Therefore, the CEST method provides a practical way to monitor the germination of bacterial spores. Due to the complexity of a bacterium and the scope of the present study, we did not focus on identifying the biochemical contributors to the apparent CEST contrast. Instead, we considered the apparent endogenous CEST contrast as the sum of CEST signals of all proton-exchangeable components of a bacterial cell

(Figure 1a), using the general description “bacterial CEST” (bacCEST). The bacCEST contrast allowed monitoring of spore germination and visualization of bacterial homing to tumors. Using LPS-induced inflammation, we verified that the increase of CEST contrast after spore injection was unlikely to be caused by the host response to the bacteria in the early stage rather than the bacteria themselves homing in to the tumor.

The bacCEST method described here, like other endogenous CEST approaches (45,49,65,66) may be confounded by physiological and pathological changes unrelated to the bacteria themselves. Micro-environmental factors including pH, temperature, the properties of the local tumor cell matrix, and the metabolic condition of tumor cells may all affect the change of the apparent CEST signal at 2.6 ppm through the alteration of proton water exchange rates (k_{ex}) and the water T1 relaxation time and content (M_0). All these factors have to be taken into account when a quantitative measurement is required. This may become particularly complicated in the late stages of bacteriolytic therapy. Tumor cell death, from either necrosis or apoptosis, is expected to result in an acidic local pH, which may influence the apparent CEST contrast in the region of interest (74). However, our results showed that neither changes in pH, LPS-induced sterile inflammation, nor necrosis of tumor cells following LPS administration significantly affected CEST contrast. These observations indicate that the present method may be sufficiently specific for detecting bacteria at early times after infection. With the aid of new acquisition schemes (75), and enhanced data post-processing (76), we expect that the specificity of bacCEST can be further improved through the elimination of unwanted competing endogenous magnetization transfer effects and interference from direct water saturation.

The immediate value of bacCEST lies in its potential to assess the success of *C. novyi-NT*-based therapy through the early detection of germination. It may be possible to use the basic principles of bacCEST to monitor therapeutic responses of tumors to other bacteria. Investigations in this area will be facilitated by *in vitro* screening of clinically relevant bacterial strains using our high throughput CEST imaging methods (56). Constructing bacCEST libraries from various bacteria will not only provide insight into the source of the CEST contrast but also serve as a repository of information for the diagnosis of bacterial infections in general.

CONCLUSION

We have explored the use of bacCEST contrast to detect bacteria, and applied this technology to monitor germination of the therapeutic bacterium *C. novyi-NT* in an experimental tumor model. This technology has the potential to be used for non-invasive monitoring of bacterial infections without the need for exogenous contrast agents.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We are grateful to Dr. Jiadi Xu and Ms. Kazi Ahkter for help with MRI experiments and Dr. David Huso for help with histology. This research was supported by NIH grants R01EB015032, R01EB012590, R21EB015609, R21EB008769, R21EB005252, and P50 CA062924, and by BioMed Valley Discoveries, Inc. and the Virginia and D.K. Ludwig Fund for Cancer Research. Dr. Bettgowda was supported by the Johns Hopkins Clinician Scientist Award and the Burroughs Wellcome Career Award for Medical Scientists.

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Figure 1. CEST MRI detection of *C. novyi-NT*

a A cartoon showing the structure of the spore form (left) and, after germination, vegetative form (middle) of bacteria. A bacterium contains a variety of molecules, either inside the cytoplasm or on the cell surface, that carry labile protons in exchange with water protons (right), resulting in marked CEST contrast (bacCEST). **b, c** CEST contrast displayed using z-spectra (**b**), and MTR_{asym} plots (**c**) of 6×10^6 bacterial cells/mL and 4×10^7 spores/mL in PBS. The arrows point to 2.6 ppm, the offset producing the highest CEST contrast for *C. novyi-NT*. **d** CEST contrast of samples as a function of bacterial density in cell culture broth (10% FBS, pH=6.5), obtained by subtracting the CEST contrast of bacterial samples from that of culture broth only, **e** the pH dependency of CEST contrast for 6×10^7 bacterial cells/mL in culture medium.

Figure 2. CEST MRI of spore germination *in vitro*

a) Photo of tubes with different spore concentration after 12 hours of ‘incubation’ inside the MRI scanner at 37 °C; After 6–7 hours, the gels in spore-containing tubes broke as a result of the pressure from production of hydrogen gas by bacterial growth. **b)** T2w images (top left quarter) showing the tube arrangement with concentration indicated by number of spores in millions. Other quarters: CEST images (MTR_{asym} maps at 2.6 ppm) of *C. novyi-NT* spores as a function of time (4.0, 5.1 and 5.7 hours) after incubation. **c)** Relative change in CEST contrast for four samples, displayed as $\Delta MTR_{\text{asym}} = MTR_{\text{asym}}(t) - MTR_{\text{asym}}(0)$, where $MTR_{\text{asym}}(t)$ is the mean CEST contrast at 2.6 ppm at time t after incubation, and $MTR_{\text{asym}}(0)$ is that measured at the start of incubation. **b** and **c** show that CEST contrast is dramatically elevated in the spore-containing sample samples after germination but not the control sample or before germination.

Figure 3. In vivo CEST MRI detection of *C. novyi-NT* germination in CT-26 murine colon tumors

a) MR images 12 hours post-injection of spores in a representative mouse. From top to bottom: T2-weighted (T2w) images of the tumor (T), CEST maps (MTR_{asym} at 2.6 ppm), and merged images showing the co-localization of segmented CEST contrast of tumor region on the T2w image. **b)** H&E and gram stains of the tumor region; H&E on the left panel indicates necrotic regions (N) in the center and remarkable inflammatory cell infiltration on the periphery, while the gram stain on the right panel shows the presence of *C. novyi-NT*, scale bar=200 μm . **c)** Comparison of CEST signals in the tumor ROI before and 12 hours after injection, including z-spectra (right y-axis scale) and MTR_{asym} plots (left y-axis scale), **d)** Histograms of CEST contrast of whole tumor ROI before (blue) and 12 hours after (red), and **e)** Comparison of the mean ROI MTR_{asym} (2.6ppm) before and after *C. novyi-NT* injection (n=6). Note that there was one outlier showing decreased CEST contrast post-injection.

Figure 4. CEST MRI of LPS-induced sterile inflammation in murine CT-26 colon tumors
a) Example of MR images of tumors before and 24 hours after intra-tumoral injection of LPS. From top to bottom: T2w images, CEST maps (MTR_{asym} at 2.6 ppm), and merged images showing the co-localization of segmented CEST contrast of tumor region on the T2w image, **b)** H&E stain (left: 2x and right: 20x) of the tumor region showing severe hemorrhagic necrosis in the tumor center (left panel) and inflammatory cell infiltration at the periphery (yellow arrows, right panel). **c)** Comparison of CEST signals in the tumor ROI before and 24 hours after LPS-injection, including z-spectra (scale on right y-axis) and MTR_{asym} plots (scale on left y-axis), **d)** Histogram analysis of CEST signal of tumor ROI before (blue) and 24 hours after injection (red), **e)** Comparison of the mean ROI MTR_{asym} (2.6ppm) before and after LPS injection ($n=4$).