# ZnDPA-DOTA Targeted Contrast Agents for MRI Detection of Bacteria in a Mouse Model of Infection

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**Background:** Bacterial infections, both localized and disseminated (bacteremia), are responsible for significant morbidity and mortality in the community and hospital settings[1,2]. Identification and localization of the bacterial source are important diagnostic challenges that are incompletely addressed by currently available imaging techniques as they lack specificity for detecting infection[3,4]. A robust method to noninvasively identify and localize pathogenic bacterial sources with high sensitivity and specificity would significantly advance the clinical evaluation and management of bacterial infections. Previously, we reported the synthesis and *in vitro* testing of two MRI-compatible contrast agents [ZnDPA-DOTA-amide (ZnDPA-DOTA-AM) and ZnDPA-DOTA-thiourea (ZnDPA-DOTA-TU)], consisting of a ZnDPA (targeting moiety) attached to a $Gd^{3+}$-DOTA ($T_1$-relaxing moiety) via an aminohexoxy spacer[5] as targeted MRI contrast agents for detecting bacterial infections. *E. coli* were incubated with varying concentrations of ZnDPA-DOTA-AM and ZnDPA-DOTA-TU conjugates in saline. *In vitro* characterization showed a strong concentration-dependent reduction in $T_1$ following incubation of the targeted conjugates with *E. coli*, reflecting probe binding as well as enhanced relaxivity due to restricted rotational motion. In the present study we report the use of these conjugates for the detection of bacterial infection *in vivo*.

**Materials and Methods: Animal model:** Four to six-week old female Balb/c mice were obtained from Jackson Laboratory (Bar Harbor, ME, USA). All experimental procedures were performed on the animals according to a protocol approved by the Institutional Animal Care and Use Committee. ***In vivo* prototype testing:** *E. coli* labeled with 1 mM ZnDPA-DOTA-TU (100 µL) and unlabeled *E. coli* in saline (100 µL) were injected intramuscularly into the target side (right flank) and control side (left flank) of the mice respectively. **Animal preparation:** During bacterial injection and *in vivo* MRI experiments, mice were anesthetized with 1.0–1.5% isoflurane in 1 L/min oxygen administered through a nose cone. For MRI, the head of the mouse was secured in an in-house developed restraining device to minimize motion induced artifacts. Subdermal needle electrodes, respiration pillow and a thermister were connected to a small animal monitoring device (SA Instruments, NY, USA) to monitor vital signs including the electrocardiogram, respiration and core body temperature. During the scan, the animal body temperature was regulated at 37±1°C by blowing warm air into the magnet bore via a hose connected to a thermostatically controlled warm air device (SA Instruments, NY, USA). **MRI:** *In vivo* imaging was performed on horizontal bore 4.7 T magnet interfaced to a Varian Direct Drive console (Palo Alto, CA, USA). A series of inversion recovery images were generated using the T One by Multiple Read Out Pulses pulse sequence[6] (TOMROP). The following imaging parameters were used: slices = 2, slice thickness = 1 mm, FOV = 5 cm, acquisitions 1, nominal flip angle = 10º, TE = 3.15 msec TR = 10 msec, NTI = 60, matrix size = 128 × 128 and total acquisition time was 25 min. **Data analysis:** $T_1$ maps were generated from the TOMROP data using a three-parameter nonlinear least-squares fit of the pixel intensity as described previously[7]. In the *in vivo* $T_1$ maps containing the site of infection, a region of interest (ROI) was manually drawn around the right flank and the left flank of the mice and the mean $T_1$ values were measured.

**Results: Figure 1** shows *in vivo* images of a mouse injected with *E. coli* in saline into the left flank and *E. coli* incubated with 1 mM ZnDPA-DOTA-TU into the right flank. In the ROI from unlabeled bacteria, the average $T_1$ values (0.373±0.067 sec) were found to be significantly ($p<0.001$) higher than the average $T_1$ values (0.125±0.017 sec) in the ROI from bacteria labeled with ZnDPA-DOTA-TU. These $T_1$ values are the average of five experiments. *E. coli* incubated with ZnDPA-DOTA-TU reduced $T_1$ by a factor of 3, indicating binding of the contrast agent to bacteria. **Figure 2** displays a representative histogram of the $T_1$ map (as shown in Figure 1) of bacteria labeled with ZnDPA-DOTA-TU and unlabeled bacteria in saline. In the bacteria labeled with ZnDPA-DOTA-TU marked by red lines, pixel distribution was between 0.1 sec and 0.2 sec, while in the unlabeled bacteria in saline, marked by green lines; it was 0.4 sec to 0.5 sec indicating a clear delineation between the $T_1$ values in both ROIs (**Figure 2**).

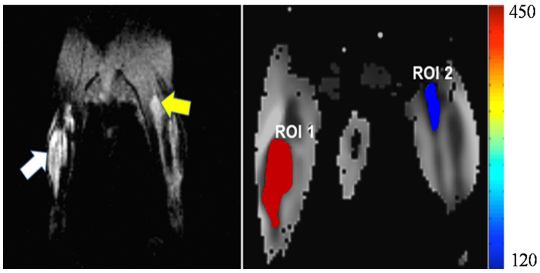


**Figure 1. Left:** Representative $T_1$-weighted images of a mouse injected with *E. coli* in saline into the left flank (white arrow) and *E. coli* incubated with 1 mM ZnDPA-DOTA-TU into the right flank (yellow arrow) **Right:** $T_1$ maps, ROI 1: *E. coli* in saline, $T_1$ = 0.427±0.090 sec; ROI 2: *E. coli* + ZnDPA-DOTA-TU, $T_1$ = 0.143±0.084 sec.

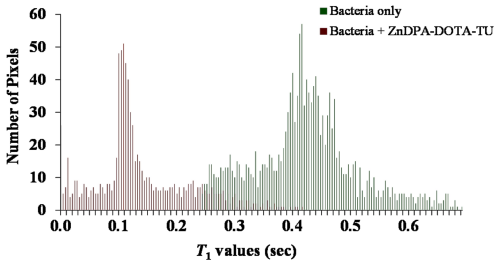


**Figure 2.** Representative histogram of a $T_1$ map (as shown in Figure 1) generated from a mouse injected with *E. coli* incubated with 1 mM ZnDPA-DOTA-TU (red lines), (count = 838, mean±SD = 0.143±0.084, min = 0.002, max = 0.420) and *E. coli* incubated with saline (green lines), (count = 1543, mean±SD = 0.427±0.090, min = 0.245, max = 0.695).

**Discussion:** Previously, we reported the synthesis of two MRI contrast agents that target bacteria with high sensitivity and specificity, utilizing ZnDPA conjugates that bind to cell surface anionic phospholipids. ZnDPA has strong affinity for phosphatidylserine and phosphatidylglycerol, with $K_D \sim 10^5$ - $10^7 M^{-1}$, making these compounds ideal for bacterial sensors[8,9]. The promise of these conjugates as *in vivo* MRI contrast agents rests upon multiple factors that relate to inherent sensitivity and specificity: high anionic phospholipid target density on bacterial membranes ($10^6$ - $10^8$ per cell), high surface to volume ratio of bacteria ($10^2$ - $10^3$ fold greater than mammalian cells), high affinity of the binding ligands, and potential further enhancement of relaxivity due to restricted molecular motion upon binding. This would indicate that focal accumulations of bound $Gd^{3+}$ 1-2 orders of magnitude greater than the minimum detectable concentration are possible, with high anticipated specificity relative to eukaryotic cells that express comparatively small numbers of binding sites. *E. coli* exposed to ZnDPA-DOTA-TU reduced $T_1$ by a factor of three indicating binding of ZnDPA-DOTA-TU to bacteria in an animal model of localized bacterial infection. In an animal model of infection, our experiments demonstrate the feasibility of enhanced *in vivo* visualization of bacteria which has been labeled with a novel contrast agent. These studies demonstrate that detection of targeted bacteria in an animal model is feasible via changes in $T_1$ relaxation. In conclusion, this study will be useful to develop these contrast agents for detection of bacterial infections in humans with high specificity and sensitivity.

**References:** [1] Rello J, et al. Clin Infect Dis 1992; 15: 184. [2] Modol J, et al. Clin Infect Dis 2002; 35: 899-900. [3] Roth AR, et al. Am Fam Physician 2003; 68: 2223-2228. [4] Palestro CJ, et al. Radiographics 2000; 20: 1649-1660. [5] Warczyk A, et al. Proc Int Soc Mag Reson Med 2009; 17: 3122. [6] Brix G, et al. Magn Reson Imaging 1990; 8: 351-356. [7] Pickup S, et al. Magn Reson Imaging 2004; 19: 508-512. [8] Leevy WM, et al. Chem. Commun. (Cambridge, U. K.) 2006; 1595-1597. [9] Leevy, WM, et al. J. Am. Chem. Soc. 2006; 128: 16476-16477.