MRI-Contrast Agents for Detection of Bacterial Infection

A. Warczyk[1,2], A. Popov[2], S. Pickup[2], A. Goldberg[2], and E. J. Delikatny[2]

[1]Pharmacology, University of Pennsylvania, Philadelphia, PA, United States, [2]Radiology, University of Pennsylvania, Philadelphia, PA, United States

Introduction. Bacterial infections, both localized and disseminated (bacteremia), are responsible for significant morbidity and mortality in the community and in hospital settings. Identification of a bacterial infection in the acute clinical setting, as well as localization of a bacterial source in known bacteremia, are important diagnostic challenges that are incompletely addressed by current radiologic methods. These methods include broad CT scanning, which relies on contrast generated by the tissue's immune response to detect occult bacterial sources. This contrast is neither sensitive nor specific. The ability to noninvasively identify pathogenic bacterial sources with high sensitivity and specificity would greatly aid in the timely diagnosis and treatment of bacteremia.

We have developed two MRI-compatible gadolinium contrast agents that selectively bind the outer bacterial cell membrane, potentially allowing for the *in vivo* detection of gram-negative and -positive bacteria with high sensitivity and specificity. The structure of these compounds is a macrocyclic DOTA chelator (octadental, $DOTA_8$ or heptadental, $DOTA_7$) linked to a bis-(zinc dipicolylamine) (ZnDPA) moiety via a zeta-aminohexanoxyphenylidene-3,5 spacer. The ZnDPA moiety binds with high affinity to anionic phospholipids, which are a major component of bacterial cell membranes, but are not present on the surface of mammalian cells[1]. The design is based on fluorescent ZnDPA constructs previously used for small animal optical imaging of infection[2,3,4]. The high surface target density and surface area to volume ratio of bacteria coupled with the high affinity of the binding agent (>10^7 M^{-1}) suggests that these agents will provide sufficient T_1-weighted contrast for detection of bacterial lesions *in vivo*. In this report we present relaxation data from bacterial cultures that demonstrate the binding properties and longitudinal relaxation rate enhancement of these targeted contrast agents.

Materials & Methods. ***Bacterial prep:*** *E. coli* were obtained from the DNA sequencing center in 150 µl pellets in microcentrifuge tubes. Solutions of contrast agent in saline at different concentrations were added to the bacteria. The cells were suspended by pipetting followed by vortexing, incubated at 37°C for 15 min, and spun at 4000 RPM for 5 min. The supernatant was removed and the cells rinsed twice with saline to remove excess contrast agent. The cells were resuspended in 50 µl saline for imaging. ***MR imaging*** was performed on a 4.7 T INOVA scanner (Varian Inc., Palo Alto, CA) using a 70 mm TEM coil (Insight NeuroImaging, Worcester, MA) containing a polystyrene holder for 12 microcentrifuge tubes. A series of inversion recovery images was generated using the T One by Multiple Read Out Pulses sequence (TE: 4 msec, TR: 10 sec, NTI: 100, FOV: 8 cm, thickness : 3 mm, matrix size: 256x128, flip angle: 20°, total acquisition time 21.5 min), a saturation recovery sequence employing a nonselective composite inversion pulse followed by NTI samplings of the same line in *k*-space using a gradient echo. ***Image analysis:*** T_1 maps were generated from the resulting images using a three parameter non-linear least squares fit of the pixel intensity as described previously[5]. Mean T_1 values from each tube were generated from ROI s that were manually drawn in the T_1 maps using ImageJ.

Results and Discussion. T_1 maps (**Figure 1**) showed water and saline controls to have R_1 values of 0.38±0.08 sec^{-1}, whereas solutions of 0.5mM Gd^{3+} DTPA (Magnevist Injection™) had an R_1 value of 3.18±0.36 sec^{-1}. Solutions of the ZnDPA-$DOTA_8$ and -$DOTA_7$ contrast agents (0.5 mM) had R_1 values of 10.12±1.45 and 6.42±0.21 sec^{-1} respectively. *E. coli* incubated in saline had an R_1 of 3.87±0.55 sec^{-1}, similar to bacteria incubated with 0.5 or 1 mM Magnevist (R_1=4.22±0.56 or 3.78±0.43 sec^{-1} respectively). The fact that the R_1 observed with Magnevist incubation was not different from saline incubation indicates that the Gd^{3+} is completely removed by rinsing and does not bind to the bacteria. *E. coli* incubated with the two novel contrast agents showed a four-fold increase in R_1s resulting from the high affinity binding with bacterial membrane phospholipids: 21.20±6.02 and 14.27±2.27 sec^{-1} for 1 mM ZnDPA-$DOTA_8$ and -$DOTA_7$ respectively (**Figure 2**). This R_1 increase was linearly concentration-dependent and detectable as low as 0.125 mM. This indicates that these contrast agents are of great potential use for further *in vivo* investigation.

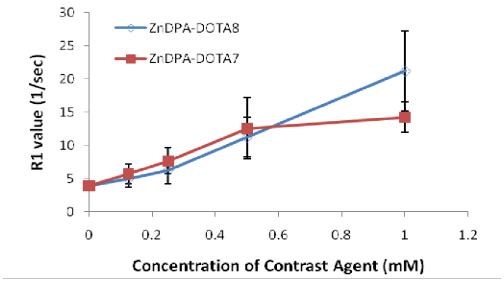

Figure 2 Concentration dependence of R1 value demonstrates four-fold increase with addition of 1 mM of either contrast agent (ZnDPA-$DOTA_8$ or -$DOTA_7$).

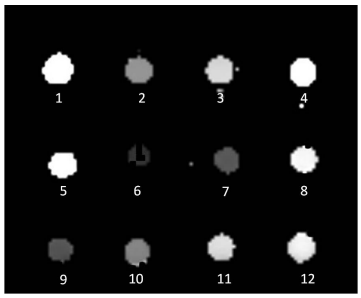

Figure 1 A typical T_1 map generated in the current study. (1: 0.5 mM Magnevist; 2: 0.5 mM ZnDPA-$DOTA_8$; 3: 0.5 mM ZnDPA-$DOTA_7$; 4: bacteria in saline; 5: bacteria incubated with 1 mM Magnevist; 6-8: bacteria incubated with 1.0, 0.5 and 0.25 mM ZnDPA-$DOTA_8$, respectively; 9-12: bacteria incubated with 1.0, 0.5, 0.25, and 0.125 mM ZnDPA-$DOTA_7$, respectively.)

References: 1. A. Ojida et al, *JACS*, **124**, 6256, 2002; 2. A. Koulov et al, *Cell Death Differ.*, **10**, 1357,2003; 3. W. Leevy et al, *Chem. Comm.*, 1595, 2006; 4. W. Leevy et al, *JACS Comm.*, **128**, 16476, 2006; 5. S. Pickup et al, *JMRI*, **19**, 508, 2004.