Developing a Pipeline of Bacteria-Specific Imaging Agents

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Early accurate diagnosis of infection is essential for effective therapy, but traditional diagnostic methods are invasive, labor intensive and time consuming, as well as subject to the uncertainties of incorrect sampling and contamination. CT and MRI detect anatomic changes that occur late in a disease process and are neither sensitive nor specific for the diagnosis of bacterial infections. Moreover, though more sensitive, nuclear medicine imaging (99Tc-tagged WBC or [18F]FDG-PET) have poor specificity in differentiating between sterile inflammation and infection. Therefore, bacteria-specific imaging tracers are required to discriminate infection from other disease processes, and to monitor treatment efficacy.

We hypothesize that small prokaryote specific molecules can be identified and developed for use as radiotracers. We screened a commercial library of over 400 random 14C and 3H radiolabeled small molecules looking for low molecular weight compounds with excellent penetration into diseased tissues and scored these molecules according to our selection criteria: metabolized by prokaryote-specific pathways, evidence for prokaryote accumulation or antimicrobial activity, and absence of known eukaryotic accumulation or metabolism. Compounds of the library that passed all 3 selection criteria and were tested for intracellular bacterial accumulation in model bacteria representing three important pathogen classes: *Staphylococcus aureus* (gram-positive), *Escherichia coli* (gram-negative), or *Mycobacterium smegmatis* (mycobacteria). Intracellular bacterial accumulation was determined by % of cell associated radioactivity measured at different time points using a scintillation counter.

Seven of the eight compounds were accumulated within *E. coli*, with sorbitol and D-xylose selectively retained by this organism. 4-Aminobenzoic acid (PABA) and D-mannitol were noted to accumulate in all species of bacteria significantly and rapidly. Follow up testing of PABA with *M. tuberculosis* revealed 96%±15% cell associated radioactivity at 18 hours of incubation. *In vivo* PET imaging was performed using a sorbitol analog (2-[18F]fluorodeoxysorbitol - FDS) showing a significant difference between infection and inflammation in a murine myositis model. Our findings were extended to models of mixed Gram-positive and Gram-negative thigh coinfections, brain infection, *Klebsiella* pneumonia, and mice undergoing immunosuppressive chemotherapy.

We have developed an innovative approach for screening bacteria-specific imaging tracers with promising results including [18F]-FDS which is a candidate imaging probe for translation to human clinical cases of known or suspected infections owing to Enterobacteriaceae. These tools would be useful in both preclinical and clinical settings for a broad variety of bacterial infections, including tuberculosis in which could be a key component for decision making and appropriate treatment.