**Gadolinium is a Good Antimicrobial Surrogate for In Vivo Imaging**

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**Introduction:** It is well understood that antimicrobials elute from antimicrobial loaded bone cement (ALBC) based on their chemical properties, loading, and the porogen content of the cement. Bench-top studies evaluate the period of time over which antimicrobial release is detectable from the vehicles, sometimes in reference to the MIC of target organisms. The critical piece of information missed by these studies, however, is how the release from the depot translates into concentration of antimicrobial in surrounding tissues \textit{in vivo}. While a depot may be releasing antimicrobials for a long period of time, the flux of antimicrobial may not be enough to overcome the tissue clearance of that antimicrobial to build up a therapeutic concentration and manage residual biofilm and bacteria in the surgical wound.

Recently, there has been interest in the use of contrast based MRI to image local delivery of antimicrobial surrogates in surgical wounds. There is also considerable controversy as to the effectiveness of local delivery in infection management. Imaging of gadolinium distribution is based on the assumption that gadolinium and vancomycin have similar delivery properties and will travel together through tissues affected once locally delivered. While it is reasonable based on the molecular weight and solubility of these molecules to presume they will behave similarly, this project seeks to test that assumption at both the release interface, and in the tissues after release. This project asks two questions: 1. Do vancomycin and gadolinium release similarly from bone cement in vitro and 2. What is the in vivo distribution of gadolinium released from bone cement also loaded with vancomycin?

**Methods:** Simplex P\(^\circ\) Bone Cement (Stryker, Mawah, NJ) was hand mixed under a sterilization hood without negative pressure. The mixture included Poly methyl methacrylate powder 10g, mmA 5mL, 945mg vancomycin-HCL (Hospira Inc., Lake Forest, IL), 250mg Gd-DTPA (Sigma Aldrich, St Louis , MO), 1136mg K2HPO4, and 169mg KH2PO4. The K2HPO4 and KH2PO4 were sieved to a size between 250 and 425 microns before being mixed into the cement. As the cement was hardening, it was molded both into a custom Teflon template to form 1x1cm cubes and also into red rubber catheters (Covidien, Mansfield, MA, USA) to form rods with a diameter of 4mm. In addition to the catheters and cubes, cylindrical molds were filled to create 12mm x 6mm diameter rods (ASTM F451-08). All cement was allowed to harden for 24 hours prior to machining. Cubes were removed from the mold without machining, although a rodent was used to remove rough edges prior to sterilization. Rods were physically snapped, and then had the red rubber removed with a razor blade prior to sterilization. Cylinders were machined flat and smooth with a low speed saw prior to elution. Sterilization was performed via autoclaving or ethylene oxide exposure, where appropriate. Three cylindrical cement depots were then placed in 15 mL of phosphate buffered saline (PBS) in vials in triplicate and stored at 37\(^\circ\)C. The rods were transferred to fresh PBS at time points 1hr, 5.5hr, 24hr, and 48hr. A high performance liquid chromatography (HPLC) machine was used to detect the amount of vancomycin and gadolinium in each vial averaged over the three cylinders. The HPLC was run isocratically at 1 mL/min with a mobile phase of 8:92 acetonitrile:water with 0.2% triethylamine, pH 3. Detection was performed at 220 and 280 using a Diode Array Detector and area under the curve (AUC) was calculated to determine concentration of eluant. A standard curve corrected to active weight of vancomycin and gadolinium was constructed for each eluant. Vancomycin was detectable to 1 \(\mu\)g/mL. Gd-DTPA was detectable only down to 100 \(\mu\)g/mL. Cumulative mass release was calculated by summing release over time, and converted to molar release using the formula weight reported by the manufacturer. Release was compared at 1 and 5.5 hours using t-test.

After a New Zealand white rabbit was anesthetized with ketamine/xylazine and maintained with 2% isoflurane, an anterolateral approach to the femur was made with a #15 blade scalpel. On the left lower extremity, the dissection was carried down to the mid shaft of the femur and 1gm of muscle tissue was removed with a rongeur. A 1mm bur was then used to create a cortical defect in the bone. This was a trough shaped defect that measured 1cm in length and involved 50% of the circumference of the bone. Next, two bone cement rods as mixed above in the elution protocol were inserted into the intramedullary canal of the femur through the cortical defect. One implant slid proximally and the other distally. On this extremity, the deep muscle and fascial layers were not primarily repaired but the skin was closed with 4-0 prolene suture (Ethicon, Cincinnati, Ohio) to serve as our full thickness wound model (figures 2,3).

On the right lower extremity, the incision was carried down to the mid shaft femur and a rongeur was used to remove approximately 1 gm of quadriceps muscle tissue. A cube of bone cement as mixed above was then inserted into the resulting potential space. After implantation of the cement depot, the superficial muscle, fascia lata and skin were closed in layers with 4-0 prolene suture. The right lower extremity served as our partial thickness wound model (figures 2,3). All procedures were compliant with the National Institutes of Health guidelines for the care and use of laboratory animals and approved by the...
In vivo images were taken daily for six days using a Bruker Biospin® 7-T MRI (Bruker Biospin, Billerica, MA). 42 axial fat-suppressed T1-weighted rapid acquisitions with relaxation enhancement (RARE) images were collected of the thighs from hip to knee. Each slice was 2mm thick, with voxel sizes of 0.3 mm x 0.3 mm x 2 mm. A series of these T1-weighted MRI images at four repetition times (TR) (1463, 2000, 3000, and 5000 ms) were obtained. Previously published image processing techniques were used to produce concentration maps and 3D projections of the volume covered by delivered antimicrobial.

**Results:** Vancomycin and Gd-DTPA were both detectable within the first 5 hours of the release study. Vancomycin and Gd-DTPA molar concentration were similar at 1 hour (p=0.84, t-test), and at 5 hours (p=0.99). Gd-DTPA concentrations fell below the detectable limit of 100 ug/mL after 5.5 hours and so no additional comparisons were performed.

Co-delivery of vancomycin and Gd-DTPA produced drug distributions that were qualitatively similar to those seen previously for 2g Gd-DTPA with 8g of xylitol. Daily imaging of the drug delivery in both legs indicated that delivery became undetectable within 48-72 hours. Example 3d reconstructions are shown at day 1 and 5 (Figure 1).

**Discussion:** Our in vitro data suggest that when loaded in equal molar concentrations, gadolinium and vancomycin have similar delivery properties from bone cement. This was expected based on their chemical structures and relative solubility. While the gadolinium could not be detected after the 5.5 hour time point, enough of the curve was captured to indicate that the release of the two molecules was similar.

The in vivo portion of our experiment showed that gadolinium release is not only detectable from bone cement via MRI imaging, but also that the release is unchanged compared to prior studies that did not also load the bone cement depots with vancomycin. This data implies that gadolinium could be co-delivered with vancomycin loaded bone cement determine post-implantation drug distribution in future clinical trials.

**Significance:** Gadolinium can be used as a marker for antimicrobial release and distribution from bone cement with MRI imaging.
Figure 1: Sample MRI 3D reconstructions, anteroposterior view. Visual differences over time in the distribution of Gd-DTPA are exemplified by these images. The blue coloration marks areas of >50% of Gd-DTPA. The rabbit sustained a left femoral fracture on post-operative day #2.

References:

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