Depth and Duration of Antimicrobial Tissue Penetration Depends on Dose: A Pilot Study with Gadolinium

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Statement of Purpose:

Drug release from antibiotic loaded bone cement is frequently studied on the bench top in a variety of conditions. It is difficult to relate these experiments to performance *in vivo*, however, because different boundary conditions exist for different clinical applications. Similarly, clinical studies show variations in outcomes like infection rate based on presumed differences in antimicrobial delivery. This study seeks to join the bottle to the bedside.

The study asks two questions: 1. Do vancomycin and gadolinium release similarly from bone cement in vitro? 2. What volume of tissue has a concentration above 14 ug/mL of antimicrobial surrogate 7 days after depot implantation?

Methods:

Cement formation: 10 g Simplex P® Bone Cement was hand mixed sterilely without vacuum with 250mg Gd-DTPA, ___ mg Vancomycin and 1.2 g sodium phosphate, sieved to 250-425 µm. In dough phase, the cement was molded into a custom Teflon molds to form 1x1cm cubes and 12mm x 6mm diameter cylinders (ASTM F451-08). Cement was also molded into red rubber to form rods with a diameter of 4mm. All cement was allowed to harden for 24 hours prior to machining. Cylinders were machined flat and smooth with a low speed saw.

Elution: Three cylindrical cement depots were then placed in 15 mL PBS in vials in triplicate and stored at 37^{o}C. The cylinders were transferred to fresh PBS at time points 1hr, 5.5hr, 24hr, and 48hr. Isocratic HPLC was used to detect of vancomycin and gadolinium. Detection was performed at 220 and 280nm. Vancomycin was detectable to 1µg/mL. Gd-DTPA was detectable at 100 µg/mL. Cumulative mass release was calculated and converted to molar release. The release profile was compared at 1 and 5.5 hours using t-test.

Animal Model: 1 cm cubic Gd-DTPA loaded bone cement (GLBC) local delivery implants were made with either 1g, 4g, or 10g of Gadolinium- diethyl triamine pentaacetic acid powder (Gd-DTPA) per 40 g PMMA powder. No additional poragen was used. Implants were placed in surgically created soft tissue defects in NZW rabbit quadriceps. 1g of muscle tissue was removed to create the defect. Muscle and skin were closed over the implants. Wounds were imaged on a 7T Bruker MRI with a RARE T1 weighted sequence at 1 day and 7 days. To eliminate the T2 star distortions from the contrast in the implant. Prior to imaging on day 7, incisions were opened and depots were replaced with control depots containing no contrast. After imaging, original depots were replaced. T1 weighted images at serial TR ranging from 1500 to 5000 were used to construct a T1 map of the tissue of interest at each time point. Volume of distribution was reconstructed using Mimics and MATLAB, and compared between dosages using ANOVA.

Results:

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.

A large difference in volume of distribution was seen based on dose (2100mm^3 for 10g vs 600-700mm^3 for 4 and 1g) (p=0.002) (Fig 1). Contrast was generally gone by 7 days from ALBC with a 1g load. Tissue that had been exposed to 4g GLBC had high local concentrations initially, and some tissue penetration remained by 7 days. Tissue exposed to 10g GLBC had much larger volumes of distribution at 7 days.

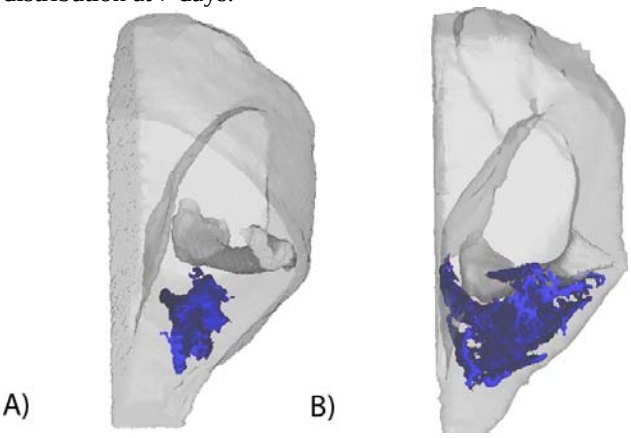

Figure 1: Volume of Distribution from Implants of Various Dosages at 7 Days. The blue region shows the region of contrast and implant distributed in the leg of a rabbit. The femur is shown in white. A) shows the distribution from a 1cm cubic implant from1 g/batch Gd-DTPA cement mixture. B) shows the distribution from a 1cm cubic implant with 10g/batch of Gd-DTPA.

Conclusions:

The higher the initial loading of Gd-DTPA the further it penetrated tissue and longer it was retained above a threshold value. The threshold value was equivalent to the concentration of antimicrobial that might be required to treat biofilm residing microbes. Therefore, we can extrapolate that clinically the volume of tissue that retains antimicrobial at therapeutic levels for more than a few days is highly dependent on the load of antimicrobial placed within the depot.

Furthermore, Gadolinium-DTPA can be used as a marker for antimicrobial release and distribution from bone cement with MRI imaging.