

## Telavancin and Vancomycin Elution and Efficacy after Addition to Bone Cement

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**DISCLOSURES:** This project is funded by Theravance Biopharma.

**INTRODUCTION:** Periprosthetic joint infections (PJI) is one of the most challenging problems in orthopedic surgery. One form of PJI begins with adhesion of bacteria to the implant and the production of a biofilm from the bacterial strains [1,2]. Some of these bacterial strains such as staphylococci and gram-positive cannot be completely eliminated by antibiotics and cause necrosis of soft and hard tissues surrounding the implant. Antibiotic-loaded bone cement (ALBC) may be an effective option to prevent PJI. Local administration of antibiotics can reach higher levels of drug concentration, than can be reached by systemic administration [3]. However, the addition of antibiotics may weaken the cement's mechanical properties. In vitro, vancomycin (VAN) added to Palacos R bone cement has demonstrated sufficient drug elution to be effective against main pathogens. Also, the mechanical properties of Palacos with VAN meet the minimum ISO requirements. Telavancin (TLV) is an investigational lipoglycopeptide antibiotic, effective against gram-positive pathogens, and is anticipated to have similar results as VAN when added to cement. In this study, two different antibiotics (VAN and TLV) with two types of cements (Palacos R and Simplex P) were compared in vitro for drug elution and efficacy and cement mechanical properties.

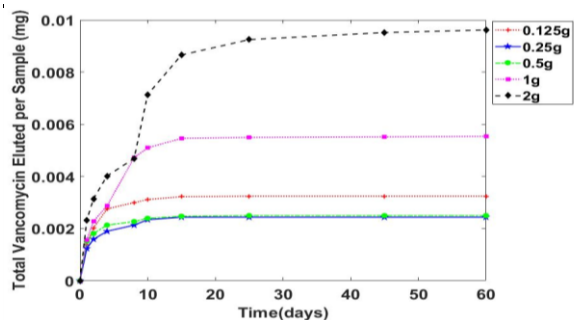
**METHODS:** Four types of ALBC's were prepared with two types of bone cement, Palacos R (Heraeus Medical GmbH, Wehrheim, Germany) and Simplex P (Stryker Corporation, Kalamazoo, Michigan, USA) Two different antibiotics, VAN (Sigma-Aldrich, St. Louis, MO) and TLV (Theravance Biopharma) to investigate drug elution and efficacy; flexural strength and modulus; compressive yield strength and modulus; and, fracture toughness. Samples were prepared as previously described [4] in accordance with clinical method and ISO testing standards. Antibiotic elution tests were carried out for 60-days and the eluted mass was quantified at regular intervals using high performance liquid chromatography (HPLC). Individual samples were placed in 5 mL of phosphate-buffered saline (PBS), stored in incubator 37°C and shaken at 100 rpm. For each time point, 1.5 mL of PBS solution was taken from the 5 mL of PBS solution, and stored at -80°C. HPLC testing was performed on 10  $\mu$ L of the PBS solution. Antibiotic efficacy was measured against four common bacterial strains (two methicillin-resistant *S. aureus*: n315 and ATCC 33591, one methicillin-susceptible *S. aureus* ATCC 29213 and one *S. epidermidis* ATCC 35984). The 6 mm diameter by 4.5 mm high cylindrical samples were submerged in 3.5 mL of tryptic soy broth (TSB), inoculated with bacterium, and the bacterial suspensions were serially diluted in saline on Mueller-Hinton agar (MHa). The MHa plates were incubated for 18 to 24 h at 37°C and bacterial concentrations were quantified. Drug efficacy tests were performed in triplicate for each time point, over a two-week period. Mechanical testing of properties in four-point bending, compression and single-edge notched beam fracture toughness tests were conducted according to ISO standard test methods, previously described [4]. Mechanical testing was conducted on an electromechanical materials testing frame (Criterion C43.103, MTS systems, Eden Prairie, MN, USA) with force and displacement data collected at 100 Hz. Fracture surfaces were imaged with scanning electron microscopy (SEM). Mean and standard deviations for all mechanical properties were calculated for statistical analysis with Minitab 18(Minitab Inc, State College, PA).

**RESULTS SECTION:** HPLC testing of the drug elution samples found 2.0 g TLV added to Palacos or Simplex bone cement samples produced the highest eluted mass. 0.2 % of the incorporated TLV eluted from Palacos over the 6-8 week period (**Fig 1**); whereas, 0.07% of the TLV eluted from Simplex. The highest percentage of drug released from Palacos was 1.03% with the addition of 0.125 g of TLV. This result was in contrast to only 0.10% as the highest percentage of drug released from Simplex with the addition of 1 g of TLV. The percentage of TLV eluted from either cement was significantly less than that of vancomycin. The eluted dosages from samples with 2.0 g VAN per Palacos or Simplex package were sufficient to eliminate a 10<sup>3</sup> CFU/mL inoculum of *S.aureus* organisms within 10 days. However, 2.0 g of TLV from Simplex was not able to fully clear the initial inoculum of four tested strains (**Fig 2**). Adding more than 0.5 g of VAN or TLV per Palacos package reduced compressive yield strength to below the ISO 70MPa minimum. Flexural strength and fracture toughness were not significantly affected by the addition of either antibiotic.

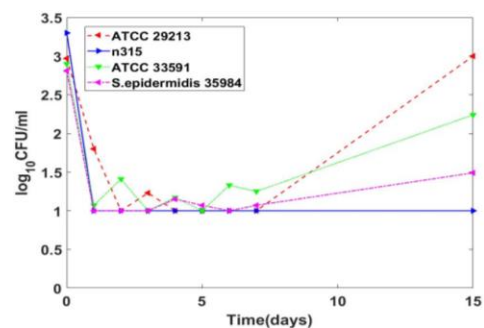
**DISCUSSION:** The samples with 2.0 g of TLV in Palacos produced the highest mass of eluted antibiotic whereas 1.0 g of VAN samples were the highest mass of eluted antibiotic. The elution of both antibiotics was significantly less from Simplex than Palacos cement. The difference in elution between the two types of cement may be due to difference in pore size and number. VAN eluted from Palacos was effective in eliminating 10<sup>3</sup> CFU/mL inoculum in the two tested MRSA strains, but was significantly less effective at eliminating *S.epidermidis* strain which has high biofilm producing characteristics. TLV eluted from Simplex was not able to fully clear the inoculum of the four Staphylococcus strains. Mechanical properties were significantly affected by the addition of antibiotics, perhaps due to the influence of pores. TLV and Simplex samples tended to have larger pore sizes, and lower mechanical properties.

**SIGNIFICANCE/CLINICAL RELEVANCE:** VAN eluted from Palacos was effective in eliminating 10<sup>3</sup> CFU/mL inoculum in the two tested MRSA strains, but was significantly less effective at eliminating *S.epidermidis* strain which has high biofilm producing characteristics. TLV eluted from Simplex was not able to fully clear the inoculum of the four Staphylococcus strains. Adding more than 0.5 g of VAN or TLV per cement package reduced compressive yield strength to below the ISO 70MPa minimum.

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**Figure 1:** 1 to 60 days elution data of Telavancin from Palacos bone cement disk. N=15 at each data point



**Figure 2:** Activity data for 2.0 g of Telavancin added per package of Simplex cement with  $10^3$  initial CFU.