Amphotericin B Delivery from Antimicrobial Loaded Bone Cement is Increased by Poragens

[1]Kweon, C; [1,2]McLaren, A; [2]Leon, C; [1]Miller, R; [1, 2]McLemore, R
[1]Banner Good Samaritan Medical Center, Phoenix, AZ 85006, [2]Center for Interventional Biomaterials, Arizona State University, Tempe, AZ 85287

Statement of Purpose:

Amphotericin B is reported to not be released from acrylic bone cement in elution studies but is detectable in wound fluid from amphotericin B ALBC in clinical use. The compressive strength of the amphotericin B ALBC in those elution studies was reported to be higher that control, contrary to conventional understanding that antimicrobial powder weakens cement. Amphotericin B has low solubility and forms micelles in aqueous salt solutions.

Previous research by McLaren and others report increased antibacterial release when porosity of ALBC is increased using poragens and compressive strength of low-dose ALBC is initially increased above control but decreases with time in elution, leading to the study questions- Is amphotericin B released from ALBC? Is amphotericin B release from ALBC increased by adding a poragen? Does amphotericin B increase the compressive strength of ALBC and does the compressive strength of amphotericin B ALBC decreases with time elution?

Methods:

ALBC Cylinder Preparation:

Test cylinders were made from two formulations of ALBC prepared using 200mg of amphotericin B deoxycholate (Xgen Pharmaceuticals Big Flatts, NY) with 1) no poragen or 2) cefazolin 10g (Apotex Pharmaceuticals, Toronto, ON) per batch of Simplex® acrylic bone cement (Stryker Mahwah, NJ). Test cylinders measuring 12 mm x 6 mm diameter (ASTM 451-99) were prepared in a Teflon mold. The ends of the cylinders were machined square and flat for length precision and mechanical testing.

Elution:

5 cylinders of each ALBC formulation were individually eluted in 2 mL of deionized water at 37°C maintaining infinite sink conditions. Total eluant exchange was performed at 1, 3, 7, and 15 days. Amphotericin B in the eluate was extracted with 50 vol% dimethyl sulfoxide (DMSO) (Sigma-Aldrich St. Louis, MO) to disperse the micelles. Amphotericin B concentration was measured with UV spectrometry at 415 nm using a Fluostar Omega UV spectrophotometer (BMG Labtech). Amphotericin B calibration curves were constructed over a range of cefazolin concentrations to account for UV absorbance from cefazolin. Cumulative recovered amphotericin B, M_t, was calculated and the data was analyzed with Repeated Measures ANOVA.

Compression:

The compressive strength of both formulations was tested before elution, and after 1 and 30 days of elution. 5 cylinders for each formulation were eluted in 25 mL of DI water at 37°C. Total eluant exchange was carried out on days 1, 3, 7, and 15. The test cylinders were towel dried and stored in sealed glass scintillation vials until testing. All test cylinders were loaded to failure in axial compression at 24.0 mm/min using an MTS Syntech 1/S mechanical testing machine (MTS Systems, Eden Prairie, MN). Load-displacement data were analyzed using a custom MATLAB (The Mathworks Inc, Natick, MA) algorithm to determine compressive strength in accordance with ASTM Standard F451-99.

Results:

M_t from the ALBC with no poragen over 15 days was 1.66 µg and from the ALBC with 10 g cefazolin as poragen was 12.76 µg ($p<0.001$, Repeated Measures Anova). Compressive strength of ALBC without poragen was 88 MPa before elution, (control with no antimicrobial or poragen, 75 MPa), with minimal change over time in elution to 94 MPa and 97 MPa after 1 and 30 days of elution respectively. For ALBC with 10 cefazolin as poragen, compressive strength was 80 MPa before elution, decreasing with time in elution to 61MPa and 46 MPa after 1 and 30 days of elution respectively (both: $p<0.001$, t test).

Conclusions:

Amphotericin B is released from acrylic bone cement, and consistent with soluble, hydrophilic antibacterials, release is limited when the PMMA encases the amphotericin B load. This is consistent with 3.2µg/mL in wound drainage report by Marra. However amphotericin B forms micelles. Amphotericin B micelles are not detectable by UV spectroscopy at 415um and micelle formation is highly affected by salt concentration. The gentamicin sulfate loaded in the acrylic bone cement with the amphotericin B reported by Goss likely drove the released amphotericin B into micelles explaining why there was no measurable amphotericin B. As with antibacterial loaded bone cement, when poragen is used to increase porosity, more of the drug load is released. In the case of amphotericin B, extraction with DMSO facilitates its assay however a soluble non-salt poragen such as dextrose may be a better choice than the cefalozlin used in this study. The hydrophobic nature of the PMMA vehicle may attract the amphotericin B as it is released creating a barrier layer on the cement surface, further limiting detection of amphotericin B released from PMMA. The initial increase in compressive strength seen in the formulation without a poragen is consistent with that which occurs with low-dose antibacterial ALBC, and maintaining that increase in strength over time in elution may be related to cross-linking by amphotericin B. The decrease in strength over time in elution seen in the formulation with 10 gm of cefazolin is consistent with the degradation in strength seen from poragen dissolution in high-dose antibacterial ALBC.