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INTRODUCTION

Antibiotic tolerant biofilms play an important role in the pathogenicity of chronic periosteal joint infections (PJIs). Local administration of antibiotics as vancomycin (VAN) and tobramycin (TOB), alone or in combination are commonly used in the treatment of PJIs. Staphylococcus aureus and Pseudomonas aeruginosa are common biofilm forming pathogens in PJIs.

Antibiotics can be released from polyethylene melt-cast (PMMA) bone cement spacers and antibiotic-loaded calcium sulfate beads to achieve high local concentrations of antibiotics for extended periods of time required to significantly kill biofilm bacteria. Antibiotic-loaded calcium sulfate (ALCS) Stimulant Rapid Cure) has shown promising results for eradication of these biofilms in-vitro. Antibiotic-loaded PMMA bone cement (AL-PMMA) and powdered antibiotic sprinkled in the surgical site as a bolus (PAB) are additional strategies used to achieve high local concentrations of antibiotics. Antibiotic concentrations in the joint space using these different administration methods is poorly understood. Conventional release testing relies on elution into a fixed liquid volume and is not compatible with a bolus application. Also, in PJIs there is liquid exchange from joint fluid and exudate. Here we describe an in-vitro reactor flow system, to better determine the predicted antibiotic concentration profile from these release strategies.

METHODS

- A reactor flow system was used to pump Ringers solution simulating clinical values of post-surgical drainage after knee arthroplasty revision.
  - The flow rate was initially set to 3.5mL/min and was frequently changed to align with clinical values. From 48 hours onwards the flow rate was reduced to 0.02mL/min.
  - A PMMA bone cement spacer (40g Simplex) was added to the model which was either unloaded (PMMA) or loaded with 2000mg VAN and 2000mg of TOB (AL-PMMA).
  - ALCS beads were loaded with VAN and TOB at 100 and 200 mg/10cc respectively (ALCS).
  - The powdered antibiotic bolus (PAB) was achieved by adding 1000mg VAN powder directly to the reactor flow system immediately prior to initiating flow.
  - Effluent samples (2.5 mL) were collected, and antibiotic potency was semi-quantitatively assessed using the Kirby-Bauer method of zone of inhibition.
  - Zones of Inhibition (ZOI) were performed against S. aureus UAMS-1 and P. aeruginosa PAO1 to confirm the potency of antibiotic released over time.
  - Bacteria (1 X 10^6 CFU/mL) were spread onto tryptic soy agar (TSA). Paper discs (6mm) were placed equidistant from each other onto the previously spread TSA plate.
  - 10µL of the effluent was spotted onto the corresponding discs and incubated for 24 hours.
  - The area of each ZOI was determined using NIH imageJ.
  - When the culture grew right up to the filter, it was interpreted as the effluent had no antimicrobial potency.

CONCLUSIONS

- ZOI analysis showed an increased killing of S. aureus and P. aeruginosa and longer elution by ALCS compared with AL-PMMA and PAB.
- Additionally, ZOI testing showed increased killing with ALCS in combination with AL-PMMA than with AL-PMMA alone.
- The PAB bolus (measured as potency against UAMS1) initially had the highest immediate potency but was rapidly washed out to below potency within less than 2 hours.
- The AL-PMMA alone showed initial potency but was washed out by 1 hour, however the potency increased at 16 hour when the flow rate had slowed allowing the antibiotic concentration to build back up.
- ALCS alone or in combination with a loaded spacer (ALCS + AL-PMMA) provided improved potency over an extended period compared to AL-PMMA or a PAB bolus alone.
- The reactor system has potential to more accurately predict how local antibiotic administration from cement, ALCS or a PAB bolus may be optimized to treat biofilms associated with PJIs.
- Limitations of the study: in vitro elution and potency kinetics of antibiotics is dependent on the specifics of the system. Lack of soft tissue removes a potential source for retaining antibiotic.

RESULTS

Z0I of effluent from Reactor Flow System analysed against S. aureus (UMBAS).

Figure 3A: Short-term analysis showing rapid decrease in VAN levels in PAB as compared to AL-PMMA and ALCS + AL-PMMA.

Figure 3B: ZOI analysed for 6 weeks showing an increased killing of S. aureus and longer elution by ALCS + AL-PMMA than AL-PMMA and PAB.

Figure 3C: Short-term analysis showing greater ZOI was observed with ALCS + AL-PMMA than AL-PMMA alone.

Figure 3D: ZOI analysed for 6 weeks showing an increased killing of P. aeruginosa and longer elution by ALCS + AL-PMMA than AL-PMMA.

DISCUSSION

Based on these in-vitro results, it is evident that ALCS beads loaded with VAN/TOB provide an extended release at above inhibitory concentration for longer than a single bolus (PAB) and also provide an attenuated effect when used in combination with AL-PMMA. Further work is required to confirm results.

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