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In vitro susceptibility of *Propionibacterium acnes* to simulated intrawound vancomycin concentrations

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Background: There is convincing evidence supporting the prophylactic use of intrawound vancomycin powder in spinal fusion surgery and mounting evidence in the arthroplasty literature suggesting that it can reduce surgical site infections. As a result, a number of shoulder arthroplasty surgeons have adopted this practice, despite a paucity of evidence and the presence of a pathogen that is, for the most part, unique to this area of the body—*Propionibacterium acnes*. The purpose of this study was to evaluate the efficacy of vancomycin against planktonic *P. acnes* in vitro, using time-dependent concentrations one would expect in vivo after intra-articular application.

Methods: Intrawound vancomycin concentrations were interpolated and extrapolated from existing in vivo data. Planktonic *P. acnes* was then subjected to a time-kill analysis during 96 hours. At each time point, the inoculum was centrifuged into pellet form and then reconstituted for serial drop counts onto blood agar plates. After anaerobic incubation, colony-forming units were counted, and $\log_{10}$ colony-forming units per milliliter were determined.

Results: Early time points grew to confluence, and thus colony-forming units per milliliter were not calculated. However, at 12 hours of vancomycin treatment, distinct colonies were appreciated. Notably, there was a $3 \times \log_{10}$ reduction in colony-forming units per milliliter between 12 and 48 hours, denoting bactericidal activity. In addition, *P. acnes* was completely eradicated after 3 days of treatment.

Conclusion: When administered in a fashion meant to simulate time-dependent in vivo intrawound concentrations, vancomycin exhibited bactericidal activity against *P. acnes*. This may lend credence to the prophylactic use of vancomycin in shoulder surgery.

Intrawound vancomycin powder application has been used extensively in posterior instrumented thoracolumbar fusion, and there is good evidence to support its utility in the prevention of postsurgical wound infections in this context.[1,20] This makes intuitive sense as >60% of clinical isolates from surgical site infections in the United States are resistant to cephalosporins, including methicillin-resistant *Staphylococcus aureus* as well as coagulase-negative *Staphylococcus* species such as *Staphylococcus epidermidis*.[20] Vancomycin, in general and despite the emergence of drug-resistant strains, is still considered to be effective against most strains of these pathogens.

It is a small conceptual leap to expect a similar decrease in surgical site infections in the context of intrawound vancomycin application and total joint arthroplasty, given that the predominant clinical isolates from infected joints are similar to those found in the spine.[7,9] However, the evidence for this in the existing arthroplasty literature is not as convincing, despite mounting evidence. A recent retrospective study has, in fact, shown a statistically significant reduction in early prosthetic joint infections in the context of revision total knee and hip arthroplasty after intrawound vancomycin application.[14]

Hoping that these results may translate, a number of shoulder arthroplasty surgeons are now using intrawound vancomycin powder to prevent prosthetic joint infection. The evidence in support of this is even more sparse than that which exists in the hip and knee arthroplasty literature; however, some have reported that its use is both clinically useful and cost-effective in the reduction of infection in shoulder arthroplasty.[8] Although this may be true, the predominant clinical isolate found in prosthetic shoulder arthroplasty infections, *Propionibacterium acnes*, is different from those found in the realm of hip and knee arthroplasty and spine surgery.

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In a study evaluating the antibiotic susceptibility of planktonic forms of *P. acnes* clinical isolates, the activity of vancomycin was found to be fair, with a minimum inhibitory concentration (MIC_{50}) of 0.38 μg/mL.[3] These authors go on to note that this nominal activity is especially relevant in the context of biofilm formation, as the vancomycin concentration required to eradicate *P. acnes* biofilms has been previously reported as ≥128 μg/mL.[3,6,17]

In a knee and hip arthroplasty study, the time-dependent intrawound concentration of locally applied vancomycin was estimated on the basis of the concentrations found in closed drains.[10] Not surprisingly, the concentration profile as a function of time observed in that study is accurately modeled as a first-order process.[4,10,11,19] It is our hypothesis that the initial concentrations and rate of absorption and elimination of vancomycin in the shoulder will be comparable to those found in the hip and knee. In this study, and based on the data provided by Johnson et al,[10] we estimated the intrawound vancomycin concentration from 0 to 96 hours postoperatively. We then performed a time-kill study during this interval using planktonic *P. acnes* exposed to vancomycin at these simulated concentrations. Our ultimate goal was to evaluate the effectiveness of vancomycin against *P. acnes* under conditions we would expect in vivo.

Materials and methods

Determining estimated intrawound vancomycin concentrations for in vitro time-kill study

Data from Johnson et al[10] were analyzed, and a linear equation was formulated on the basis of assumed first-order pharmacokinetics and exponential decay (Fig. 1, *A*; $R^2 = 1$). This equation, in turn, was used to calculate the intrawound vancomycin concentration at any point in time. As the in vitro component of this study was not continuous but instead separated into 3-hour blocks, the time-averaged concentration was used in the time-kill study (Fig. 1, *B*).

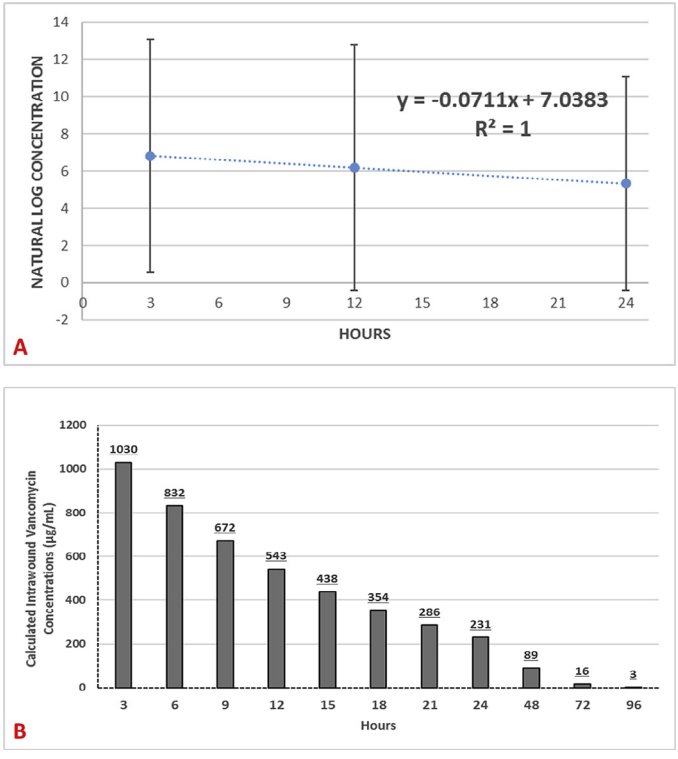

Figure 1 First-order treatment of previous intrawound vancomycin concentrations achieved by Johnson et al[10] yielded a linear equation with $R^2 = 1$ (**A**). With this equation, vancomycin concentrations at any point in time can be calculated and used in vitro to simulate in vivo conditions (**B**).

Time-kill study

A clinical isolate of *P. acnes* in a chopped meat glucose broth anaerobic vial (Anaerobe Systems, Morgan Hill, CA, USA) was obtained from Banner University Medical Center Phoenix. Using a serologic pipette, 0.2 mL of chopped meat glucose broth from the bottom of the vial was transferred into 3 mL of brain-heart infusion (BHI) broth (Alpha Biosciences, Baltimore, MD, USA). This was subsequently placed in a 2.5-L AnaeroPack chamber (Mitsubishi Gas Chemical Company, Tokyo, Japan) and incubated at 38°C until turbid. For both experimental and positive control samples, 0.75 mL of the resulting *P. acnes* inoculum was placed in microcentrifuge tubes to achieve n = 2 for all time points.

Vancomycin hydrochloride (Sagent Pharmaceuticals Inc., Schaumburg, IL, USA) standard concentrations were prepared in autoclaved BHI. Vancomycin concentrations prepared included 2060, 832, 672, 543, 438, 354, 286, 231, 89, 16, and 3 μg/mL.

To all the experimental samples containing 0.75 mL of *P. acnes* inoculum, 0.75 mL of the 2060-μg/mL vancomycin solution was added to achieve 1.5 mL of inoculum with a vancomycin concentration of 1030 μg/mL. The positive controls were also brought to 1.5 mL total volume by adding 0.75 mL of autoclaved BHI. All samples were placed back in the anaerobic chamber and allowed to incubate for 3 hours at 38°C.

At 3 hours, the samples were collected. All samples were centrifuged for 5 minutes at 5000 rpm. A stable bacterial pellet was formed. The remaining fluid was decanted from all samples using a micropipette. For the 3-hour experimental sample, the pellet was washed to remove residual antibiotic and then reconstituted in 1.5 mL of BHI in preparation for drop counts (see later). For the future experimental samples, the pellet was reconstituted in 1.5 mL of the next (lower) concentration. For all control samples, the pellet was reconstituted in 1.5 mL of fresh BHI. The future experimental samples and positive controls were placed back in the anaerobic chamber until the next time point. This process was repeated at 6, 9, 12, 15, 18, 21, 24, 48, 72, and 96 hours.

At any given time point, 200 μL of the appropriate reconstituted experimental sample was transferred to a 96-well tissue culture plate (Celltreat; Cole-Parmer China, Shanghai, China), and serial 1:10 dilutions were performed. A 10-μL volume of the original sample and each serial dilution was then dropped onto a trypticase soy agar plate with 5% sheep blood (Becton Dickinson, Sparks, MD, USA). This process was repeated for the appropriate reconstituted positive control. Experimental samples and positive controls from the same time point were placed on the same blood agar plate and then placed back in the anaerobic chamber for incubation × 3 days at 38°C. This process was repeated for all time points.

After 3 days of anaerobic incubation, the plates were retrieved and colony-forming units were counted directly (Fig. 2). If all the serial dilutions formed a lawn of growth not amenable to counts, it was marked as confluent. Otherwise, colony-forming units per milliliter were calculated as Count * Dilution factor * 100. The $\log_{10}$ colony-forming units per milliliter were plotted against time. Data were analyzed using single-factor analysis of variance. A single post hoc 2-tailed *t*-test was used to evaluate for significant differences in colony-forming units per milliliter between time intervals thought to represent potential bactericidal activity. Significance was set at $P \leq .05$.