Title:

Novel Bisphosphocin Nu3 Demonstrates Rapid in vitro Killing of Bacteria-Encased in Biofilm

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Abstract:

Background: Bacterial biofilms pose a significant treatment challenge to traditional therapies because they enable the bacteria to persist in a dormant or slow growth phase. Biofilms are increasingly becoming associated with chronic infections and implantable device failure as well as resistance of bacteria to conventional antibiotics, thus highlighting the need for more effective therapeutics. Nu3, a member of a novel class of extremely broad-spectrum antimicrobials, was evaluated for its ability to rapidly kill biofilm-encased bacteria in vitro.

Methods: The bactericidal activity of Nu3 against biofilm-encased bacterial strains of Acinetobacter baumannii, Klebsiella pneumoniae, and Staphylococcus epidermidis was evaluated using a time-kill assay to assess the post-antibiotic effect (PAE). Bacteria were grown 24 hours in tryptic soy broth with 1% dextrose (TSBD) in sets of four borosilicate glass tubes to allow formation of the biofilm on the tube wall. The medium was carefully removed and the tubes treated with 170 U/ml Nu3 for 10 min and 30 min, or sterile saline. Following the room temperature incubation, the tubes were washed with sterile saline. TSBD was added to all four tubes and the tubes were incubated at 37°C for 24 hours without shaking. After a 24-hour incubation, the cultures were visually examined for growth and appropriate dilutions were made and aliquots plated in onto TS agar plates to enumerate colonies.

Minimum inhibitory concentration (MIC), minimum bacteria concentration (MBC) and time-kill studies against the planktonic forms of these bacteria were also performed to confirm the bactericidal activity of Nu3.

Results: Nu3 exhibited a rapid bactericidal effect on biofilm-encased bacteria with a 100% kill of all four bacterial strains observed at 170 U/ml and exposure time of 10 minutes. These results further support experimental data showing Nu3 is directly bactericidal through a mechanism of action involving depolarization of the cell membrane, which is in contrast to most traditional antibiotics.

Conclusion: Nu3 displays rapid bactericidal activity against both gram-negative and gram-positive biofilm-encased bacteria, highlighting its potential as a new topical antimicrobial therapy for infected wounds or prophylactic treatment prior to surgical closure or implantation of medical devices. This project was supported in part by the Tufts Collaborates! Seed Grant Program, Tufts Provost's Office.