

Halicin and Aminoglycosides Synergize Against *Staphylococcus aureus* in Both Planktonic Cultures and Biofilms

Joash R. Suryavanshi, MD; Akira Morita, MD, PhD; Shota Higashihira, MD; James E. Slavin, MS; Dalton Walsh, BS; Roman M. Natoli, MD, PhD; Ed M. Greenfield, PhD

Purpose: Biofilms protect bacteria from antibiotics in fracture-related infections (FRIs). Halicin was recently repurposed as an antimicrobial and we found it eradicates *Staphylococcus aureus* biofilms in vitro on orthopaedic substrates like titanium alloy (Ti6Al4V). Antibiotic combinations can act synergistically. We hypothesize halicin can synergize with relevant antibiotics against planktonic cultures and biofilms.

Methods: Antibiotics conventionally utilized in orthopaedic infections were tested in combination with halicin in checkerboard assays of *S. aureus*-Xen36 planktonic cultures. Synergistic combinations (American Society of Microbiology criteria) in planktonic cultures were tested against 24-hour biofilms on Ti6Al4V discs in the presence or absence of halicin (250 μ M = MBEC/4). Minimum biofilm eradication concentrations (MBECs) were defined as the lowest concentration preventing detectable colony formation after biofilm disruption. Symbols depict medians from independent experiments, each with 4 biofilms/group. Statistical analysis was performed by Generalized Estimating Equation using SAS v9.4.

Results: In planktonic cultures, halicin synergized with tobramycin, gentamicin, and cefazolin but not with rifampicin or vancomycin. In biofilm experiments, halicin synergized with tobramycin ($P < 0.0001$) and gentamicin ($P < 0.0001$), decreasing each MBEC 8-fold (red circles in Figure). Cefazolin was ineffective against biofilms at doses 800 times its planktonic minimum inhibitory concentration, regardless of halicin addition.

Conclusion: Halicin and aminoglycosides synergize against *S. aureus* planktonic cultures and biofilms. Future studies will test these combinations in murine FRI models.

OTA Grant

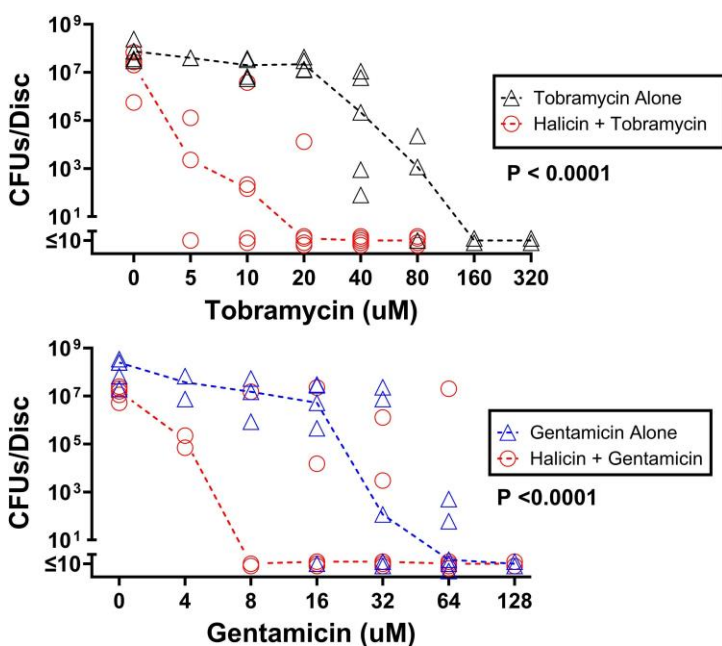


Figure: Halicin (250 μ M) decreased MBECs for tobramycin and gentamicin 8-fold.