

Effects of Halicin on S Aureus Small Colony Variants

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Purpose: Small colony variants (SCV) of *Staphylococcus aureus* cause persistent orthopaedic infections because of antibiotic tolerance and immune system evasion. Environmental stressors, including biofilm formation and subtherapeutic antibiotic exposure (especially aminoglycosides), induce these small, slow-growth colonies. Halicin, a novel antimicrobial agent, is effective against biofilms, including those formed by *S aureus* on orthopaedic implants. We hypothesized that halicin would prevent SCV emergence in *S aureus* biofilms treated with aminoglycosides.

Methods: *S aureus*-Xen36 biofilms grew on titanium alloy (Ti6Al4V) disks for 24 hours (less mature) or 7 days (more mature) and were treated with halicin and/or an aminoglycoside. Wild-type biofilm colony forming unit (CFU) counts were measured after 24 hours of growth on kanamycin-treated agar. We identified SCVs as colonies with a diameter <1 mm after 72 hours of growth on gentamicin-treated agar (2 µg/mL) to prevent overgrowth by wild-type colonies.

Results: Subtherapeutic levels of halicin monotherapy (<250 µM, one quarter of its minimum biofilm eradication concentration [MBEC]) induced accumulation of low levels of SCVs (<100 CFUs). In contrast, subtherapeutic levels of aminoglycoside monotherapy (<40 µM tobramycin MBEC, <32 µM gentamicin MBEC) induced high levels of SCVs with up to 106 CFUs per disk. Combining 250 µM halicin with either aminoglycoside inhibited SCV accumulation at concentrations that induced SCVs as monotherapy (see figure).

Conclusion: Halicin substantially reduces (>100-fold) the induction of SCVs by aminoglycosides. SCVs should be taken into account when studying antibiotics and recalcitrant infections.

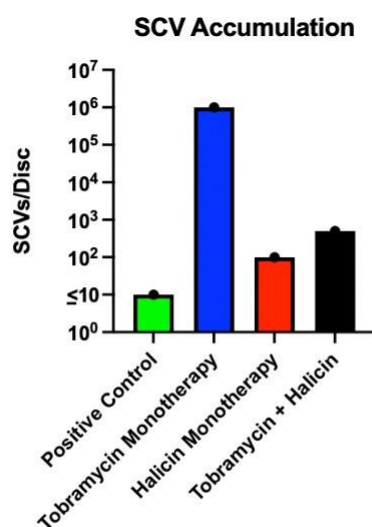


Figure 1: Sub-therapeutic halicin (<250uM) induces few SCVs as monotherapy and successfully reduces the induction of SCVs by aminoglycosides by >100-fold.