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Streptokinase treatment reverses biofilm-associated antibiotic resistance in Staphylococcus aureus in vitro

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Background: *Staphylococcus aureus* is a common cause of biofilm infections, both in native tissue and on the surface of medical implants. A key aspect of *S. aureus* pathology is its interaction with host extracellular matrix proteins, which *S. aureus* can adhere to via extensive array adhesion molecules. As antibiotic treatment of *S. aureus* biofilm is often ineffective, the host extracellular matrix – microbial adhesin interaction may be a target for improving treatment outcome.

As host matrix proteins are readily available *in vivo*, we grew *S. aureus* biofilms in the presence of human plasma to test the hypothesis that antibiotics in conjunction with the fibronolytic agent streptokinase treatment could eradicate mature biofilms with antibiotic dosages, that can safely be administered to patients.

Material/methods: EDTA stabilized human plasma in different concentrations (5,10, 25 & 50 %) was added to 3.7 % BHI and Methcillin-resistant *Staphylococcus aureus* (MRSA) strain USA300 biofilms were grown for 24 h in peg lid-based assay. Subsequently, mature *S. aureus* and MRSA USA300 biofilms were grown in 50% plasma in BHI for 24 h. Minimal biofilm eradication concentration (MBEC) dosages of vancomycin and vancomycin + rifampicin (10 mg/L) was then measured. MBEC was measured both with and without adding 500 U/mL streptokinase. As a negative control, MBEC ± streptokinase was measured for biofilms grown in tryptic soy broth without human plasma.

The efficacy of fibrinolytic and antibiotic treatment was also visualized by confocal scanning laser microscopy, using differential staining of living and dead cells, and Alexa 647-conjugated fibrinogen to visualize fibrin in the biofilm matrix.

Results: Adding human plasma to 3.7 % BHI resulted in a dose-dependent increase in biofilm biomass, with a 120-fold increase of biomass when 50% plasma is added compared to normal BHI. Immature (2 h old) biofilms could readily be removed from polystyrene peg lids (Nunc-Immuno TSP) by treating with streptokinase (500 U/mL), but after 24 h growth, streptokinase at high concentrations (>10.000 U/mL) could not remove biofilms. The MBEC value for vancomycin against MRSA strain

USA300 decreased from 128 to 64 μg/mL when adding streptokinase during the treatment. If rifampicin was included, the MBEC value in the presence of streptokinase decreased further to below our detection limit of 4 μg/mL. Treatment of *S. aureus* biofilms with streptokinase also resulted in a dramatic decrease in the MBEC value for ofloxacin from 1024 μg/mL to below the 4 μg/mL detection limit.

Conclusions: To our knowledge, this is the first study that has demonstrated that an adding streptokinase to antibiotics can reduce MBECs *in vitro* to clinically relevant levels. If similar effects can be obtained in vivo, the combination of fibrinolytic drugs with antibiotics is a powerful new approach to treat biofilm infections.