2020 CFID Undergraduate Summer Student Research Grant Final Report

Project title: Evaluating the efficacy of PslG hydrolase antibiotic combination therapy in the eradication of *Pseudomonas aeruginosa* biofilms

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Background

Individuals with cystic fibrosis (CF) are susceptible to lung infections by opportunistic pathogens such as *Pseudomonas aeruginosa* (Pa), which colonize the lung and grow as biofilms composed of self-secreted polysaccharides, DNA and proteins[1]. The protective properties of biofilms may lead to antibiotic eradication failure and chronic infection, which is associated with decreased quality of life and premature death[2]. Tobramycin is an aminoglycoside antibiotic that eliminates Pa by binding to its ribosomal subunit and inhibiting protein translation[3]. Inhaled tobramycin is an effective treatment for CF patients with Pa infections, but eradication failure still occurs in a considerable proportion of CF patients, highlighting the need to develop novel therapeutics[4]. PslG hydrolase ($PslG_h$) is an endogenously expressed enzyme involved in Pa biofilm remodelling and was first characterized by the Howell Lab at SickKids[5]. It has previously been demonstrated to improve antibiotic penetration and host immune function in laboratory and clinical Pa isolates by degrading the Psl exopolysaccharide, a molecule that facilitates cell-cell interactions and provides structural integrity in Pa biofilms[6,7]. In the present study, we investigated whether $PslG_h$ in combination with tobramycin delivered at concentrations achievable by nebulisation in CF airways results in additional biofilm reduction for 14 Pa strains isolated from the airways of individuals with CF.

Methods

14 clinical Pa isolates were obtained from a cohort of pediatric CF patients that underwent tobramycin eradication treatment at SickKids for onset Pa infections from 2011-2014. Cryogenic Pa isolates were sub-cultured on blood agar plates three times. Three biological replicates of each strain were inoculated into Luria Broth (LB) and incubated overnight at 37 °C, shaking at 225 RPM. 1:100 dilutions were performed the following day and allowed to grow until OD_{600} ~0.1. 200 uL of inoculum was transferred into each well of a microtiter plate and incubated at 37 °C for 24 hours to form biofilms. Planktonic cells were then removed with a micropipette and the pre-formed biofilms were treated with LB-infused tobramycin (1,000 ug/ml) and $PslG_h$ (0.05 ug/ml) alone and combined. After incubation for 18 hours, the remaining biofilms were stained with a 0.1 % (v/v) crystal violet solution and solubilized with 100% ethanol. Biofilm biomass was quantified spectrophotometrically at 600nm and one-way ANOVA was used to compare the means of different treatment groups. Following a similar protocol in a chambered-well model, biofilms were also imaged using confocal laser scanning microscopy to allow for a visual comparison of the different treatment groups.

Results

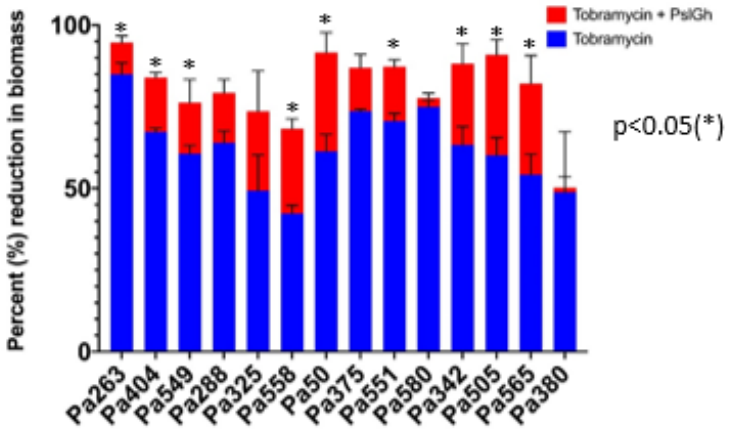

Figure 1: Percent reduction in biomass for 14 P. aeruginosa strains compared to controls. Of the 14 strains, 9 (62.3%) demonstrated a significant increase in biofilm eradication with combination treatment compared to Tobramycin alone, with no instances of antagonism observed. There was an 18.25% [12.75% - 23.76%, 95% CI] additional reduction in biofilm biomass when $PslG_h$ was combined with tobramycin. Each data point represents the mean of three individual experiments, with 8 technical replicates each.

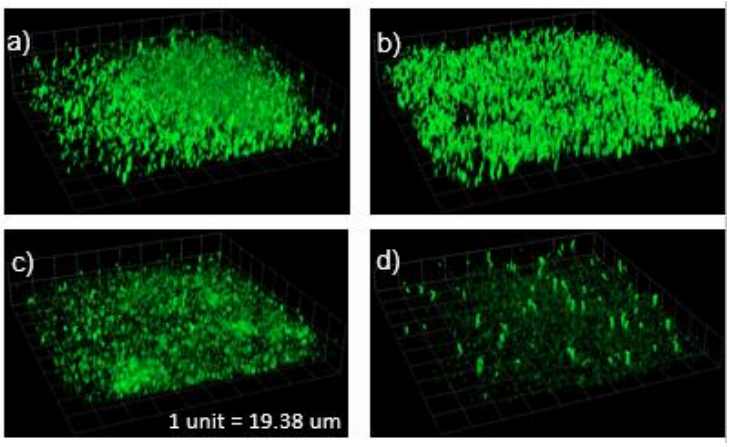

Figure 2: Representative confocal images of *P. aeruginosa* biofilm (Pa50) after treatment. (a) No Treatment, (b) $PslG_h$, (c) Tobramycin, and (d) $PslG_h$ + Tobramycin

Conclusions

Adding $PslG_h$ to tobramycin increased clearance of biofilms from CF clinical *P. aeruginosa* isolates, highlighting $PslG_h$ as a promising adjunctive therapeutic for the treatment of chronic *P. aeruginosa* infection in individuals with CF. These results validate previous findings regarding the biofilm-degrading properties of $PslG_h$ and support the development of this compound as a novel adjunctive therapeutic for Pa infections in patients with CF. Future directions include the exploration of $PslG_h$ with other antibiotics such as aztreonam, levofloxacin, and colistin.

References

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