Investigation into the Bactericidal Activity of Optochin

Introduction

Streptococcus pneumoniae is a condition-dependent α/β-hemolytic pathogen that is known to naturally colonize the upper respiratory tract of humans *(9)*. *S. pneumonaie* is the leading cause of bacterial pneumonia and meningitis globally and is especially deadly in individuals who are immune-supressed, or deficient *(3)*. Development of the pneumococcal conjugate vaccine PCV13 has significantly reduced the global burden of the pathogen in humans, but its widespread deployment has led to an increase in the amount of drug-resistant *S. pneumoniae strains* (DRSP) *(4)*.

Ethylhydrocupreine hydrochloride, widely known as Optochin, is a narrow spectrum antimicrobial introduced in 1911 which targets all four groups of pneumococci, including *S. pneumoniae (9)*. Because of its remarkable selectivity toward pneumococci, Optochin has been used in the clinical laboratory for decades as a diagnostic tool for distinguishing between strains of α-hemolytic streptococci. Although found to be effective in the treatment of pneumococcal meningitis and septicemia, development of Optochin as an antibiotic was discontinued due to toxic side effects observed in approximately 5% of patients in the form of optical disturbances when high doses were given *(2)*.

A previous SAR campaign led by the lab of Dr. Courtney Aldrich resulted in the formulation of an optochin analog (termed **48**) with 16-fold enhanced activity relative to the parent compound Optochin. Investigation from this work into the mechanism of action of Optochin utilizing **48** suggested its molecular target to be the c-ring of ATP Synthase in *S. pneumoniae*, an essential cellular component required for ATP synthesis, potentially inhibiting

growth of *S. pneumoniae* via disruption of pH homeostasis *(1,5)*. Although this work provided strong evidence regarding the target of **48** there still remain important questions regarding the mechanism of killing by this antimicrobial.

Based upon biochemical and genetic evidence, my hypothesis is that the inhibitory activity of **48** and its parent compound optochin is mediated through disruption of pH homeostasis, resulting in cytoplasmic acidification, a mechanism distinct from other ATP synthase inhibitors *(8)*. To test this hypothesis I will begin by assaying for changes in the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Activity (MBC) of **48** as a function of medium pH. We anticipate that by lowering the pH of growth medium used while culturing *S. pneumoniae* we will observe a significant decrease in both these metrics through more rapid cytoplasmic acidification, thus decreasing the amount of **48** required for activity. Following this I will construct a strain of *S. pneumonaie* expressing a pH-sensitive ratiometric green fluorescent protein (GFP) probe that will allow for direct interrogation of the effects upon cytoplasmic pH when grown in lowered pH medium to confirm our results *(6,7)*.

Material and Methods

First, I will establish the effect of acidic pH on *S. pneumoniae* growth in preliminary experiments at acidic pH ranges by setting up growth curves at physiological pH (~7.5) to acidic pH (5.5) in 0.5 increments. To test for inhibition and bactericidal activity of **48**/Optochin at decreased pH, MIC and MBC assays will be employed. To determine if decreased pH impacts the MIC of these drugs the broth microdilution method will be utilized (Clinical and Laboratory Standards Institute (CLSI)). Serial dilutions of Optochin, **48**, as well as control drugs

vancomycin and ciprofloxacin will be made across a 96-well plate with cells added. The MIC will be defined as the minimum amount of drug required to inhibit 90% of growth. MBC assays will be used to determine the minimum concentration at which bactericidal activity takes place, which will be defined as a 99.9% reduction in initial inoculum. Once the MIC has been determined, overnight cultures of S. pneumoniae with drug concentrations above the MIC will be enumerated on 5% blood agar plates to determine CFU/mL , or in other terms, the number of viable cells.

To further verify the mechanism of killing of **48**/Optochin, a recombinant strain of *S. pneumoniae* constitutively expressing a pH-sensitive GFP will be constructed using a shuttle vector specific to *S. pneumoniae* that can non-invasively measure intracellular cytoplasmic pH *(6,7)*. We hypothesize that lowered pH of the medium will increase the rate of cytoplasmic acidification and increase the bactericidal activity of **48**/Optochin.

Conclusion

Elucidating the mechanism of killing by **48**/Optochin on *S. pneumonia*e will shed light on the viability of Optochin and the synthetic analog **48** as potential inhibitors to include in *S. pneumoniae* treatment regimens. If our results indicate **48**/Optochin's inhibitory and bactericidal activity can be linked directly to cytoplasmic acidification, future directions may include conducting studies to improve the pharmacodynamic and pharmacokinetic properties and behaviour of **48** , as well as, chemical studies to produce analogs whose levels of toxicity would be suitable for inclusion in standard anti-pneumococcal regimens. From this project I will be presenting my work at Aldrich Lab meeting and may result in publication.

References

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