9. 其他佐证材料

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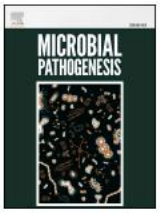

Dryocrassin ABBA ameliorates *Streptococcus pneumoniae*-induced infection *in vitro* through inhibiting *Streptococcus pneumoniae* growth and neutralizing pneumolysin activity

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Alnustone inhibits *Streptococcus pneumoniae* virulence by targeting pneumolysin and sortase A

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ABSTRACT

Streptococcus pneumoniae (*S. pneumoniae*) is a major Gram-positive opportunistic pathogen that causes pneumonia, bacteremia, and other fatal infections. This bacterium is responsible for more deaths than any other single pathogen in the world. Inexplicably, these symptoms persist despite the administration of effective antibiotics. Targeting pneumolysin (PLY) and sortase A (SrtA), the major virulence factors of *S. pneumoniae*, this study uncovered a novel resistance mechanism to *S. pneumoniae* infection. Using protein phenotype assays, we determined that the small molecule inhibitor alnustone is a potent drug that inhibits both PLY and SrtA. As essential virulence factors of *S. pneumoniae*, PLY and SrtA play a significant role in the occurrence of infection. Furthermore, evaluation using PLY-mediated hemolysis assay demonstrated alunstone had the potential to interrupt the haemolytic activity of PLY with treatment alunstone (4 μg/ml). Co-incubation of *S. pneumoniae* D39 SrtA with small-molecule inhibitors decreases cell wall-bound Nan A (pneumococcal-anchored surface protein SrtA), inhibits biofilm formation, and reduces biomass significantly. The protective effect of invasive pneumococcal disease (IPD) on murine *S. pneumoniae* was demonstrated further. Our study proposes a comprehensive bacteriostatic mechanism for *S. pneumoniae* and highlights the significant translational potential of targeting both PLY and SrtA to prevent pneumococcal infections. Our findings indicate that the antibacterial strategy of directly targeting PLY and SrtA with alnustone is a promising treatment option for *S. pneumoniae* and that alnustone is a potent inhibitor of PLY and SrtA.

1. Introduction

Streptococcus pneumoniae (*S. pneumoniae*) is a Gram-positive, facultatively anaerobic bacterium that causes severe and aggressive diseases such as pneumonia, acute otitis media, sepsis, and meningitis, particularly in children and immunocompromised individuals [1–3]. It is responsible for up to 50% of community-acquired bacterial pneumonia-related deaths, a significant diagnostic and treatment challenge [4]. It is also a leading cause of community-acquired pneumonia (CAP), accounting for 14.5 million cases and 800,000 deaths annually in children younger than five years old [5]. However, as antibiotic resistance spreads and grows, for example, over the last 40 years, resistance to penicillin and cephalosporins in strains of *Streptococcus pneumoniae* has increased globally [26], the use of antibiotics as a standard treatment for *S. pneumoniae* is threatened by rising medical costs and mortality [6]. Consequently, new therapeutic strategies to address these challenges are imminent, particularly for infections caused by *S. pneumoniae* resistant to antibiotics. In the pathogenesis of infection, *S. pneumoniae* produces several well-characterized virulence factors, such as pneumolysin (PLY), SortaseA (SrtA), capsule (Cps), pneumococcal surface protein PsaA, PspA, PspC, PavA, and PsrP, Neuraminidase, and hyaluronidase (Hyl) [7,8]. Pneumolysin (PLY), a receptor for most of the cholesterol-

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