

Towards Enhanced Efficacy of Photodynamic Antimicrobial Therapy: Insights from Murine Models Using Nebulized Delivery of Indocyanine Green-Gantrez Formulation

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Pneumonia ranks among the leading causes of death worldwide, predominantly instigated by bacteria. This bacterial infection triggers inflammation in the alveoli, leading to fluid accumulation in the lungs and respiratory difficulties. Faced with the challenge of developing and approving new antibiotics, alongside the rapid emergence of resistant strains, it is essential to pursue effective, safe treatments that do not promote resistance. Antimicrobial photodynamic therapy (aPDT) emerges as a promising alternative. aPDT has demonstrated excellent results *in vitro* and in clinical studies, exhibiting action on multiple cellular targets with high selectivity and minimally invasive characteristics. Our research has shown the efficiency and safety of a protocol for *Streptococcus pneumoniae* photoinactivation *in vitro*, as well as, light and photosensitizer delivery in animal models (Kassab et al., 2020). However, the reduction of microorganisms *in vivo* still faces challenges, primarily due to the presence of pulmonary surfactant (PS). Studies demonstrated that the photosensitizer Methylene Blue (MB) can permeate PS barriers, while Indocyanine Green (ICG) is trapped. However, when combined with the polymer Gantrez-AN 139, ICG can cross PS and completely eliminate bacteria *in vitro* in the presence of the commercial surfactants Survanta® and Curosurf®. Thus, this study aimed to evaluate, in a murine model, the efficacy of MB and the ICG-Gantrez combination using nebulization as the primary delivery method. The studies were conducted on female BALB/c AnUnib mice, approximately 8 to 10 weeks old, weighing between 20-30 g. They underwent an immunosuppression protocol and were subsequently infected with *S. pneumoniae* via nasal instillation. To assess treatment efficacy, the animals were euthanized with ketamine/xylazine overdose, followed by bronchoalveolar lavage and lung maceration to recover bacteria. Although MB possesses excellent ability to permeate PS, in our *in vivo* studies, this was not effective in eliminating bacterial strains in murine models. Fluorescence analyses demonstrated successful delivery of the formulation to its target, the lungs, yet this was insufficient for bacterial strain elimination. Conversely, ICG-Gantrez formulations exhibited significant reductions post-treatment. Fluorescence analyses at different time points indicate that both aPDT and nebulization should be administered simultaneously, as detection of the formulation is minimal after 20 minutes. Moreover, the results confirm successful formulation delivery to the lungs, underscoring the need for coordinated administration to maximize treatment efficacy. The findings of this study underscore the necessity for further research to enhance the efficacy of aPDT with the ICG-Gantrez formulation, as well as to explore new strategies and delivery methods, aiming for the complete elimination of bacterial strains.