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Exploring the potential of combined natural photosensitizers, gentamycin, and colistin in antimicrobial photodynamic inactivation of *Pseudomonas aeruginosa* biofilms

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Abstract

Background: The increasing prevalence of wound infections poses a growing threat to the healthcare system [1]. Compounding this challenge, the presence of biofilms significantly impairs both the healing process and the effectiveness of the treatment of wound infections [2]. Antimicrobial photodynamic inactivation (aPDI) has shown promising results in combating this global problem. However, its potential against Gram-negative bacteria such as Pseudomonas aeruginosa remains a major challenge [3]. Objective: The aim of this study is to investigate the effect of subinhibitory concentrations of colistin (CL) in conjugation with curcumin-gentamycin (Cur-Gen) dual combinations on boosting the aPDI of P. aeruginosa biofilms. Methods: The efficacy of CL in enhancing the photodynamic activity of Cur-Gen against biofilms was investigated using the strain P. aeruginosa ATCC 10145. Membrane permeability after CL treatment was assessed by flow cytometry. The synergistic concentrations of the triple combination Cur-Gen-CL were determined using the checkerboard assay. The efficacy of blue light (420 nm, 30 mW/cm², 10 min) to photoactivate Cur-Gen-CL and thus promote its ability to prevent and control biofilms was investigated. The total mass, metabolic activity and cell culturability of the P. aeruginosa biofilms were quantified by crystal violet, alamar blue and colony forming units (CFU), respectively. Results: Subinhibitory CL concentrations (4 µg/mL) increased the membrane permeability of P. aeruginosa by approximately 30%. This effect enhanced the efficacy of the photoactivated Cur-Gen-CL triple combination in preventing the formation of P. aeruginosa biofilms compared to Cur-Gen alone. In addition, the photoactivated Cur-Gen-CL combination achieved a complete reduction in the culturability (approximately 7 log CFU/cm²) of preformed P. aeruginosa biofilms and a reduction in biomass and metabolic activity by over 60 and 90%, respectively. Conclusions: This study has shown that the use of CL is a promising strategy to enhance the aPDI effect of the Cur-Gen combination against P. aeruginosa biofilms.

Keywords: antibiotic combinations; antimicrobial photodynamic inactivation; colistin; natural photosensitizers; *P. aeruginosa* biofilms

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