

INVESTIGACIÓN - MEDICINA

ADVANCING PHOTODYNAMIC THERAPY: COMPARATIVE STUDY OF BUTYL TOLUIDINE BLUE O AND CONVENTIONAL PHENOTHIAZINE PHOTSENSITIZERS ON FIBROBLAST VIABILITY

Claudio Teruo Kassa (claudiotkassa@hotmail.com)

Aline Souza Silva (aline.sp.br@gmail.com)

Rosani Tereza De Siqueira E Silva (rtsiqueira2015@gmail.com)

Alessandra Lima Da Silva Martins (alelima.smartins@gmail.com)

Shirlene De Holanda Lima (shirlene.holandal@uni9.edu.br)

Valherya Silva Rodriguez (svalherya@gmail.com)

Giovanna Fontgalland Ferreira (giovannafontgallandf@gmail.com)

Raquel Agnelli Mesquita Ferrari (raquelmescuita@uni9.pro.br)

Mark Wainwright (mark_wainwright@hotmail.com)

Renato Prates (pratesra@gmail.com)

Introduction: Antimicrobial photodynamic therapy (aPDT) has emerged as a promising alternative for infection control. This technique uses photosensitizers which, upon light activation, produce reactive oxygen species capable of

inactivating microorganisms. While toluidine blue O (TBO) and methylene blue (MB) are commonly employed in aPDT, a novel derivative butyl toluidine blue O (BuTBO) has demonstrated potential to improve efficacy by minimizing photosensitizer dimerization. This study aims to evaluate the effects of BuTBO, TBO, and MB on fibroblasts viability, both in dark conditions and after light irradiation. Methods: Fibroblasts were exposed to BuTBO, MB, or TBO at concentrations ranging from 100 to 0.39 μM . Viability in the absence of light was assessed after a 5 min exposure. For aPDT experiments, cells were treated with 25 μM of each photosensitizer, incubated for 5 min, then irradiated with red LED light. Cell viability was assessed by MTT immediately after irradiation and, in a separate sample, after 24 h incubation. Crystal violet was used in parallel to quantify the total adherent cell biomass, providing complementary data independent of metabolic activity. Statistical analysis was conducted using one-way ANOVA followed by Bonferroni post hoc test. Results: In the dark, all photosensitizers showed no reduction in fibroblast viability; viability remained high at concentrations $\leq 25 \mu\text{M}$. After aPDT at 25 μM , BuTBO caused a significantly greater reduction in viability compared to MB and TBO, as measured by MTT, indicating superior photodynamic efficacy. In contrast, crystal violet staining revealed no significant differences in optical density among groups, suggesting preservation of total adherent cell biomass. These data support that despite transient metabolic impairment post-aPDT, BuTBO maintains favorable cytocompatibility under the tested conditions. Conclusion: This pioneering in vitro study identifies BuTBO as an effective and biocompatible TBO-derived photosensitizer with strong potential for future clinical applications in antimicrobial photodynamic therapy.

Palavras-chave: butyl toluidine blue o (butbo); antimicrobial photodynamic therapy (apdt); 1929.