

# Mechanisms Involved in the Enhancement of Photodynamic Inactivation Mediated by Methylene Blue with Potassium Iodide

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Photodynamic Inactivation (PDI) has been largely studied as an alternative antimicrobial therapy against a range of microorganisms. PDI, which is based on the application of a photosensitizer (PS) followed by light irradiation in the presence of oxygen, results in the production of reactive oxygen species that are lethal to the microorganisms. In the attempt to enhance the PDI effectiveness, studies have shown that the addition of potassium iodide (KI) to the PS improves the inactivation ratio. Previous results of our research group demonstrated that PDI mediated by methylene blue (MB, 20  $\mu\text{M}$ ) with KI (100 mM) followed by 60  $\text{J}/\text{cm}^2$  of light irradiation (660 nm) was capable to eradicate *Pseudomonas aeruginosa* biofilm, whereas the same treatment without KI had no antimicrobial effect. One possible hypothesis is the production of iodine radicals during PDI that are toxic to the cells. The aim of the present study was to understand the mechanisms involved in the improvement of PDI effectiveness using KI. For this, the absorption spectrum of MB was evaluated (from 200 to 1000 nm) in the presence and absence of KI. Then, the MB solutions were irradiated with a 660 nm LED light device, and the absorption spectrum was followed until the full MB degradation. Additionally, the same test was performed in the absence of oxygen for comparison purposes. Then, *P. aeruginosa* biofilms were treated with iodine generated by the electrolysis of the KI, and the viability of the bacteria after the iodine exposure was measured by the colony count test. From the results obtained, the production of iodine was verified during PDI with KI in the absorption spectrum after light irradiation in the region on 230 nm. The light dose delivered for complete MB degradation was lower in the presence of KI. Additionally, the iodine production was only observed in the presence of the oxygen. The viability test showed eradication of the *P. aeruginosa* biofilm when treated with iodine radicals. In conclusion, when PDI is mediated by MB in the presence of KI, there are the production of iodine radicals that is dependent on the oxygen and the *P. aeruginosa* bacteria is susceptible to these iodine radicals.