

Photoinactivation of Bovine Mastitis Pathogens in PBS Suspensions and in Agar-Plates: Evaluation of Photosensitizer Structure and the Effect of Milk

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Palavras Chave: Antimicrobial Photodynamic Therapy (APDT); Cationic Porphyrin Photosensitizers; Bovine Mastitis Pathogens.

Highlights

The hydrophobic-hydrophilic balance present in these cationic porphyrin photosensitizers is a key factor in the interaction with the microorganisms determining the observed photodynamic efficiency

Resumo/Abstract

In this work Antimicrobial Photodynamic Therapy (APDT) was investigated in pathogens isolated from bovine mastitis employing cationic porphyrin photosensitizers (PS) in the photoinactivation of Gram-positive (*S. aureus*) and Gram-negative (*E. coli* and *P. aeruginosa*) bacteria and the microalgae (*P. zopfii*). PS of the free base *meso*-tetra-(methyl-pyridinium)-porphyrin (TMPyP - **P1**) and the respective Zn(II) complex (ZnTMPyP - **ZnP1**) were used, and the behavior of these PS compared with that of the free base *meso*-tetra-(benzyl-pyridinium)-porphyrin (TBzPyP - **P2**) and its respective Zn(II) complex (ZnTBzPyP - **ZnP2**). In PBS suspension, P2 and particularly ZnP2 are the most efficient PS in the total photoinactivation of all microorganisms isolated from bovine mastitis investigated in the present study. The greater efficiency of P2 and ZnP2 is attributed mainly to the greater incorporation and/or interaction with the microorganisms as a consequence of the structure of their porphyrinic ligands, which in addition to being tetra-cationic, have alkyl substituents that confer a greater degree of hydrophobicity to these PS compared to P1 and ZnP1. Therefore, the polar (hydrophilic) – apolar (hydrophobic) balance generated respectively by the positive charges and by the benzyl substituents, is more adequate in the interaction with the microorganisms. To evaluate the photodynamic effect in the presence of milk, photoinactivation experiments were conducted directly on agar plates to eliminate the opacity effect of the medium (milk), since only a thin film of sample applied to the plate is obtained. As a control, the behavior of the system was investigated using only PBS to apply microorganisms to the plates (Agar - PBS) and then the experiments were effectively carried out with the application of microorganisms in milk (Agar - Milk). The results on the Agar - PBS plate show the same trends as the experiments in PBS suspension, that is, P2 and ZnP2 are more effective than P1 and ZnP1, however, higher concentrations are required to observe the photodynamic effect for all PS in all microorganisms investigated. On Agar - Milk plates, P1 and ZnP1 are more effective than P2 and ZnP2 and the condition for photoinactivation that leads to the absence of viability is similar to that observed on the Agar - PBS plates. P2 and ZnP2 require relatively higher concentrations to produce photodynamic effects. This differentiated behavior on Agar – Milk plates is a clear indication that P1 and ZnP1, more hydrophilic compounds, are not affected by milk proteins and fats, while P2 and ZnP2 have a very strong interaction with the nonpolar components of milk, thus minimizing their interaction with microorganisms on the plate. ZnP1 is much more effective than P1, which may be related to its quantum yield of singlet oxygen formation, which is the highest of the series investigated. Therefore, understanding the activity of PS based on its structure and correlating it with the properties of the medium is extremely important in developing strategies for using APDT in specific problems in which infections occur.

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