Effect of $CaCl_2$ and $MgCl_2$ on the photodynamic inactivation of Gram negative bacteria by oxoaporphine alkaloid isomoschatoline

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Introduction

Photodynamic therapy (PDT) is a clinical treatment based on the combination of light, oxygen and a photosensitizer that leads to reactive oxygen species (ERO's) production and consequently the host cell death.[1] The oxoaporphine alkaloid isomoschatoline (**GB1**: Figure 1) is a blue pigment isolated from *Guatteria blepharophylla*, has gained considerable interest due its biological effects.[2] A more recent study shows the ability of the alkaloids to produce singlet oxygen and its application as a photosensitizer in PDT.[3] Studies regarding its photophysical and spectroscopic characteristics rose relevant information about application of **GB1** as a photosensitizer and photobiological studies conducted by our research group in previous work has shown photoactive activity of this compound in antimicrobial PDT with laser irradiation at 660nm and with **GB1** in sub inhibitory concentration. Gram negative bacteria are generally more resistant than gram positive due to differences in their cell wall composition that restrict the link and penetration of exterior substances. Certain additives, such as $CaCl_2$ and $MgCl_2$ may increase the permeability of gram negative bacteria outer membrane by selectively reacting with lipopolysaccharides, disrupting its integrity. Therefore, this study was undertaken to investigate the effect of $CaCl_2$ and $MgCl_2$ on gram negative bacteria photodynamic inactivation by alkaloid **GB1**.

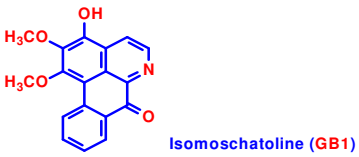

Figure 1. Structure of isomoschatoline.

Results and Discussion

Biological assay was performed employing the strain *Escherichia coli* ATCC 17099, according to the experimental procedure performed by Su et al. (2011)[4], with modifications. Treatment mixtures were prepared in a 96-well microdilution plate containing microorganism suspension (10^8 CFU/mL, Mac Farland scale), additives (final concentration of 0.05M for both $CaCl_2$ or $MgCl_2$) and **GB1** (final concentration of 50 µM). In the experiments an laser InGaAlP, model Photon Lase III (DMC®, São Carlos, Brazil) at 660 ± 3 nm, with 35 mW of output power was applied as a light source. Samples were irradiated for 5 min in an area of 0.38 cm^2 (96-well plate well area), resulting in an energy dosage of 28 J/cm^2. Control treatments without additives or, with them without light were also prepared. Treated and control microorganisms were serially diluted, plated and colonies were counted. The percentage of survivors were calculated according to the equation [(N1/N0)x100], where N0 represents the number of CFU/mL of each test sample (with or without additives) before irradiation and N1 represents CFU/mL after light exposure. Data were analyzed by determining each sample number of CFU/mL and the percent reduction of microbial growth compared to control samples. Results were expressed as means of eight replicates, with the standard error of the mean. Regarding bacterial strains, in general, gram negative bacteria are more resistant than gram positive ones and in our study, this phenomenon was also observed (when comparing growth reduction between microbiological control and treatment with irradiated samples). Therefore, in order to increase the susceptibility of *E. coli* strain, the additives $CaCl_2$ and $MgCl_2$, known to disturb gram-negative bacteria outer membrane structure, were added to the mixture of bacteria suspensions and **GB1** prior to irradiation. The bacteria incubated only with additives, in the presence or absence of light, or with **GB1**, without light, showed no significant growth reductions. However, the addition of $CaCl_2$ or $MgCl_2$ in the treatments contributed to a significant ($P<0.05$) decrease in bacterial survival (70% and 87%, respectively), when compared to the treatment with **GB1** alone, after irradiation. It is believed that the increase in membrane permeability not only allowed greater alkaloid input as well as inner cytoplasmic production of ERO's (and not at the outer membrane, as usual in gram negative bacteria) responsible for the oxidative damage that leads to cell death. Despite the positive results, further investigations are necessary to characterize how this alkaloid is internalized by the bacteria at a cytoplasmatic membrane level.

Conclusion

The use of $CaCl_2$ and $MgCl_2$ as cell permeabilizing additives increased the photodynamic inactivation of gram negative bacteria by **GB1**.

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