22.021 EVALUATION OF BACTERIOPHAGE AND PHOTODYNAMIC THERAPY AGAINST MULTIDRUG-RESISTANT PSEUDOMONAS AERUGINOSA IN A GALLERIA MELLONELLA MODEL OF SYSTEMIC INFECTION

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Introducao:

Multidrug-resistant Pseudomonas aeruginosa strains have disseminated worldwide contributing with antibiotic treatment failures encouraging the search for alternative therapeutic options against associated infections. Photodynamic therapy (PDT) is an emerging therapeutic modality that is effective against a broad spectrum of multi-resistant pathogens. It employs a nontoxic drug, termed as photosensitizer (PS), followed by irradiation with low-intensity light. After photoactivation, PS accumulated by the pathogen produces reactive oxygen species that react with biomolecules, promoting cell death. Bacteriophage therapy uses viruses capable of infecting specific bacterial strains and rapidly replicate to finally lyse the host cell and consecutively infect other bacteria. Specific bacterial receptors are necessary for phages to bind and infect the target cell. Consequently, eukaryotic cells cannot be infected and very low host toxicity is generally observed.

Objetivos:

The aim of this study was to evaluate the effect of bacteriophage and photodynamic therapies, alone and combined, against multidrug-resistant P. aeruginosa using a Galleria mellonella model of systemic infection.

Metodos:

Bacteriophage therapy (1 x 10^8 pfu/mL) and PDT were employed according to previously established protocols [i.e. lytic Myovirus (SPM-1) and PDT using methylene blue (MB, 1 mM) as PS combined with irradiation provided by a light-emitting diode (LED, λ = 662±20 nm, 30 mW/cm^2, 0.9 J/cm^2)] (Gen. Announc. 2(2): e00061-14, 2014; PLoS ONE 8(2):e55926, 2013). In brief, in vitro bacteriophage activity was evaluated against twenty-six clonally unrelated multidrug-resistant P. aeruginosa strains (including IMP-1, VIM-1, VIM-2, SPM-1, KPC-2 and GES-5 producers), using time-killing (1 x 10^5 cfu/mL) and fluorescence microscopy assays. In vivo activity of bacteriophage and photodynamic therapies were evaluated by the survival time of a systemic infection model of Galleria mellonella infected with carbapenemase-producing P. aeruginosa (1 x 10^3 cfu/mL). In vitro experiments were statistically analyzed by ANOVA and Tukey’s tests after normality and homogeneity evaluation by Shapiro-Wilk and Levene tests. In vivo statistical

analysis was performed by Log-Rank test. All experiments were performed in triplicates.

Resultados:

Significant bactericidal activity (i.e. viability reduction greater than 99.9 %) was achieved in vitro by bacteriophage (2 h, $p<0.05$) and photodynamic therapies (30 s of irradiation, $p<0.05$). Interestingly, while in vivo the effectiveness of bacteriophage therapy was confirmed (100% survival, $p<0.05$), PDT did not present statistical difference to sham control and furthermore inhibited the bacteriophage action.

Conclusão:

Bacteriophage therapy can represent an interesting alternative or adjuvant approach to control multidrug-resistant P. aeruginosa systemic infections, however, MB-mediated PDT can inhibit bacteriophage therapeutic action via unrevealed mechanisms.

Apoio Financeiro:

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Comitê de Ética:

The infection protocol of the invertebrate Galleria mellonella does not require Ethics Committee approval.