ZnPc-liposomes synthesis. Antimicrobial effects on mycobacterium multidrug-resistant tuberculosis (MDR)

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Introduction

Tuberculosis (TB) is one of the top 10 causes of death and the leading cause from a single infectious agent (above HIV/AIDS). TB disease is caused by *Mycobacterium tuberculosis* (*M. tuberculosis*), a pathogenic bacterial and spread exclusively by airborne transmission. In 85% of patients diagnosed with TB, the lung is the main target organ but the disease can spread to other parts of the body (extrapulmonary disease). In 1993, the World Health Organization (WHO) declared TB epidemic a global public health emergency; actually millions of people still continue to fall sick every year.

Photodynamic antimicrobial chemotherapy (PACT) has reemerged over the last decade as an alternative method against some resistant pathogenic bacteria. PACT presents several advantages over antibiotics; for example, absence of systemic toxicity outside the irradiated area and no resistance is developed against the PSs. Due to its remarkable features, PACT has been widely studied in the last years, and several classes of photosensitizers have been explored as consequence.

Results

In this work, was evaluated the capacity of ZnPc loaded into liposomes (DPPC-Cholesterol) to photoinactivate *M.tuberculosis* (Figure1). Liposomes were synthetized by injection method according to literature. DMSO was used as solvent for ZnPc in liposomes synthesis, which is found in the handbook of pharmaceutical excipients. Dynamic light scattering (DLS) was employed to determine the average size and polydispersity index of liposomes (see Table 1).

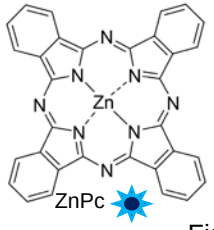
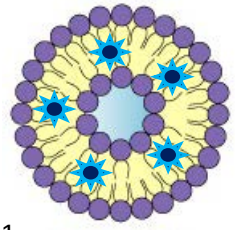


Figure1



ZnPc-liposomes

Table 1. Average size and polydispersity index of ZnPc and control liposomes synthetized by injection method

	Average size (nm)	Polydispersity index
Control-liposome	132.9	0.26
ZnPc-liposome	133.5	0.26

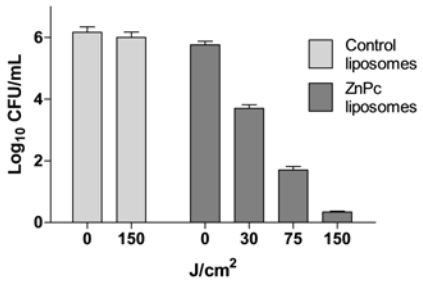


Figure 2. Photodynamic activity of ZnPc-liposomes (5.76 µg/mL) on sensible strain ATCC 27294 at different irradiation time after 2 h of incubation.

The photoinactivation efficiency was evaluated based on the decrease of the viable bacterial number (log10) in the tested and control samples. Significant photokilling effect was defined as > 3 log10 reduction in CFU/mL. Colonies forming units (CFU) on each tube were counted following 21, 28 and 40 days of incubation, as no significant changes in the results were observed, so the graphics (Figures 2 and 3) in the main text corresponds to the values of 40 days after PACT.

In Table 2, a summary of log10 decrease for both strains measured after 40 days of incubation, show the cytotoxic effect of ZnPc-liposomes and light on both strains of *M. tuberculosis*

Table 2. Photoinactivation efficiency. Log10 CFU/mL decrease after 40 days of incubation of treated bacteria with ZnPc-liposomes respect to control.

Light dose J/cm²	ATCC 27294	MDR-*M. tuberculosis* (2 h incorporation)	MDR-*M. tuberculosis* (4 h incorporation)
30	2.3	-	-
75	4.3	1.13	2.16
150	5.67	2	3.06

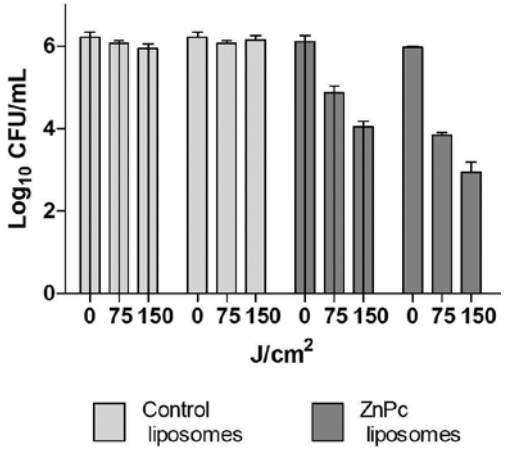


Figure 3. Photodynamic activity of ZnPc-liposomes (5.76 µg/mL) on MDR-*M. tuberculosis*. Bars with vertical lines correspond to 2 h of incubation. Bars without vertical lines corresponds to 4 h of incubation

Conclusions

We report the effectiveness of ZnPc delivered into liposomal formulation of DPPC and Cholesterol for photoinactivation of *M.tuberculosis.* Susceptible and MDR-*M. tuberculosis* strains were used in this study. Photoinactivation was subject to ZnPc effects since control liposomes no generated changes in viability cell. Susceptible strain generates more of 3 log10 CFU/mL reduction after 2 h of incubation with 75 J/cm2 or 150 J/cm2 of irradiation. Whereas, for MDR-*M. tuberculosis* was necessary adjust the incorporation time to 4 h and higher light dose to generate a decrease of 3 log10 CFU/mL. The conventional medication of MDR-*M. tuberculosis* has limited efficacy and the treatment of TB resistant has become much more complicated. Therefore, photodynamic antimicrobial chemotherapy using ZnPc-liposomes could be an alternative to treat MDR-*M. tuberculosis*, since has been shown to generate *in vitro* a decrease of 3 log10 CFU/mL or 99.9% death.