ORT.04 - Synergistic effect between silver nanoparticles and amphotericin B on pathogenic fungi

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Introduction: The incidence of severe fungal infections has increased worldwide and poses a serious global threat, especially among immunocompromised (HIV+, newly transplanted) and critically ill patients. Emergence of multidrug-resistant species also contributes to a growing mortality rate associated to fungal infections, which demands new drugs or new therapeutic strategies to manage these conditions. The use of metal ions, their nanoparticles or complexes with other binders are a promising therapeutic alternative, since it was recently demonstrated that silver nanoparticles (AgNP) possess potential antimicrobial effects in a wide range of microorganisms (bacteria, fungi and virus). Combination of amphotericin-B (AmB), a standard antifungal associated to important clinical side effects and toxicity, to AgNP could be a new promising therapeutic strategy against fungal infections.

Objective: To assess the combined *in vitro* effects between silver nanoparticles and sub-inhibitory concentrations of AmB on pathogenic fungi.

Methodology: The silver nanoparticles (AgNP) were electrochemically synthetized (18V and 500 mA), using polished silver plates in distilled water during 30 minutes at 90 ºC, and thereafter characterized and quantified by ultraviolet-visible spectroscopy and inductively coupled plasma-atomic emission spectrometry, respectively. Pathogenic fungal species, C*riptococcus neoformans* (serotype A clinical isolate H99) and *Candida albicans* (ATCC 90028), were grown *in vitro* in the presence of serial dilutions of AgNP alone or AgNP in combination with sub-inhibitory concentration of AmB (siAmB; 0.1µg/mL) during 48 hours, for assessment of fungal growth inhibition. Evaluation of yeast fungal growth was realized spectrophotometrically (592 nm). All statistical analyses were performed using one-way ANOVA followed by Newman-Keuls test. Statistics with a value of $p<0.05$ were considered significant.

Results: The concentration of electrochemically generated AgNP resulted in 18 ppm and showed no presence of silver ions in solution. AgNP alone (0.007 – 18 ppm) significantly impaired *in vitro* C. neoformans growth after 48 hours in all concentrations analyzed ($p<0.05$). Association of serial dilutions of AgNP and siAmB (0.1µg/mL) significantly potencialized impairment of *C. neoformans* growth, when compared to AgNP alone ($p<001$). In contrast, AgNP alone only inhibited *C. albicans* at 0.45, 0.9 and 18 ppm ($p<0.05$). Interestingly AgNP and siAmB *in vitro* association impaired *C. albicans* growth in all AgNP concentrations utilized ($p<0.001$). Control group using AmB (1µg/mL) completely blocked *C. neoformans* and *C. albicans* growth while siAmB (0.1µg/mL) only impaired *C. neoformans* and *C. albicans* growth (65 and 73%, respectively) after 48 h.

Conclusion: AgNP acted as an enhancing agent, potentializing the inhibitory growth effects of AmB on pathogenic fungi. Novel antifungal therapeutic strategies using AgNP, isolated or in combination, should be considered in the future.

Keywords: Silver nanoparticles; Antifungal therapeutic; Pathogenic fungi