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Effect on Antimicrobial Activity of Antifungal Agents-Silver Nanoparticles Combined Treatments

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Abstract. - *Infectious diseases are a global public health problem; they are among the leading causes of death worldwide and consume a significant amount of resources. Fungi are the most common pathogens in humans and animals. Fungal infections incidence has increased more than 200%. Currently, antifungal agents are used for combating fungal infections, but there are many problems associated with their use, such as the emergence of resistant organisms, as well as the complexity related to the development of new antibiotics. In that sense, silver nanoparticles have proven to be an alternative solution to fight fungal infections due their capacity to inhibit fungal growth. In this work, we studied the antimicrobial activity of silver nanoparticles (AgNPs), antifungal agents (Amphotericin B and Fluconazole) and combinations of both, AgNPs-antifungals over the pathogenic dimorphic yeast Candida albicans. The antimicrobial tests were performed according to the CLSI´s M27-A3 protocols. Combined AgNPs-antifungal treatments showed a different effect on antimicrobial activity. We found a synergistic effect of specific combined treatment, whereas antagonistic effect was observed with other combination.*

Keywords: Nanoantibiotics; Silver Nanoparticles; Infectious Diseases; Synergy; *Candida albicans.*

1. Introduction

Infectious diseases (ID) –one of the global first causes of death- are among the most relevant health problems worldwide, leading to social and economic issues. Also, incidence and number of ID increase every year[1]. Recently, fungal infections have increased their morbidity and mortality in immunocompromised patients, requiring intensive treatment and broad spectrum antibiotics.

Candida sp., is the most common opportunistic pathogenic fungi in humans, causing a mortality rate of up to 40 %. Currently, infection treatments are based on polyenes (i.e. amphotericin B), triazoles (i.e. fluconazole) and echinocandins (i.e. caspofungin). Nevertheless, antibiotics face several problems, such as the evolution of resistant organisms and the difficulty regarding the development of new antimicrobials. Silver nanoparticles (AgNPs) exhibit antifungal properties against both mycelial fungi and yeasts [2]. In that sense, combined AgNPs-antibiotic treatments could be useful to eliminate multiresistant microbes.

Understanding the mechanisms of AgNPs antifungal properties and synergy with antibiotics may lead to improving current treatments. In this work, we studied the effect on the antimicrobial activity of AgNPs –when combined with antifungal agents-, against *Candida albicans*.

2. Methodology

2.1 Silver nanoparticles (AgNPs)

Argovit AgNPs, functionalized with polyvinylpyrrolidone, were supplied by Investigation and Production Center Vector-Vita, Novosibirsk, Russia. Silver nitrate (Sigma-Aldrich®), was used as a reference solution.

Treatments were diluted in culture medium, on a concentration range of 0.01-1 $\mu g*ml^{-1}$.

2.2 Antifungal agents

Commercial antifungal agents were used: Amphotericin B (ApB) and Fluconazole (Flu), from Sigma-Aldrich®. For antimicrobial tests, antifungals were diluted in sterile culture medium, in a concentration range of 0.1-20 $\mu g*ml^{-1}$. Antifungals were selected according their mode of action.

2.3 Fungal strains and culture conditions

The pathogenic, dimorphic yeast *Candida albicans* strain (ATCC SC5614) was obtained from the Centro de Investigación Científica y de Educación Superior de Ensenada (CICESE). *Candida* was cultured in RPMI 1640 media for 48 h at 37 ° C, at 180 rpm (standard conditions).

2.4 Determination of Inhibitory Concentrations of AgNPs and antimicrobials in C. albicans

The antimicrobial activity of all treatments –AgNPs, $AgNO_3$, and antifungals- was performed according to the Clinical and Laboratory Standards Institute (CLSI) M27-A03 protocol, with some modifications [3]. UV-Vis spectrophotometry was used to determine partial inhibitory concentrations.

2.5 Effect of AgNPs-antifungal combined treatments on Candida´s growth

Antimicrobial activity of AgNPs-Antifungal agents combined treatments was evaluated. *C. albicans* was exposed to sub-lethal concentrations of AgNPs, antifungal agents, and the combination of these. Then, cultures were incubated into multiwell plates at standard conditions. Microbial growth was evaluated by UV-Vis spectrophotometry at λ=530 nm, in a Multiskan Go (Thermo Scientific®) spectrophotometer.

2.6 Effect of combined AgNPs-antifungal treatments on the dimorphic transition of C. albicans

C. albicans was exposed to sub-lethal concentrations of AgNPs, antifungals, and the combination of these. After cultures incubation under standard conditions, the effects on dimorphic transition was evaluated by bright-field optical microscopy.

3. Results and Discussion

The minimal inhibitory concentration (MIC) values, calculated for µg of metallic silver (active component), are shown in Table 1. We found that AgNPs MIC is like those of ionic silver and ten times lower than antifungal tested. The AgNPs MIC values reported for *C. albicans* –at similar culture conditions-, range from 10^{-1}-10^{0} $\mu g*ml^{-1}$ [4][5].

Table 1. Minimal Inhibitory Concentrations

Treatments	MIC ($\mu g*ml^{-1}$)
AgNPs	1
$AgNO_3$	0.5
Flu	10
ApB	10

Some reports have suggested that AgNPs improve the antifungal activity of antibiotics, but no reliable evidence has been provided. We found that AgNPs-antibiotics combined treatments activity was different for each antibiotic. For AgNPs/Flu, we observed an antagonistic effect, while for AgNPs/ApB, the effect was synergistic (figure 1). It is important to note that mechanisms of action of these antifungals are different; while Fluconazole's target is intracellular, Amphotericin B´s target is extracellular. We assume that changes in antimicrobial potency –in combined treatments- are due to the mechanism of action of the antifungal agents influenced by a chemical interaction between them and AgNPs. Research in this field is being performed by our group.

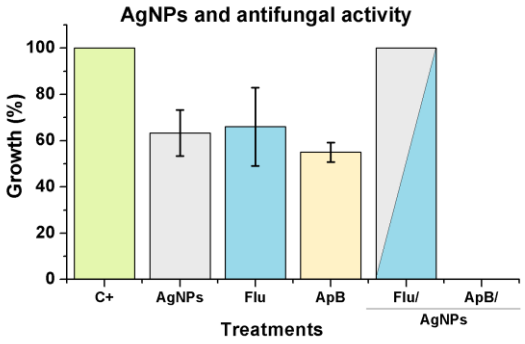

Figure 1. Antimicrobial activity of combined treatments. A synergistic effect in the AgNPs+ApB combined treatment was found, meanwhile AgNPs+Flu combined treatment showed an antagonistic effect.

We observed that none treatment influenced the dimorphic capacity of *C. albicans*. The cells treated with both AgNPs and antifungals –combined and not-combined- kept their ability to shift into hyphae or pseudohyphae (figure 2). Although it was reported that AgNPs prevent the dimorphic transition [5]. Our findings show that *C. albicans* keeps its ability to change its form, even in the presence of AgNPs. This is important to evaluate, because the hyphal phase is its pathogenic stage.

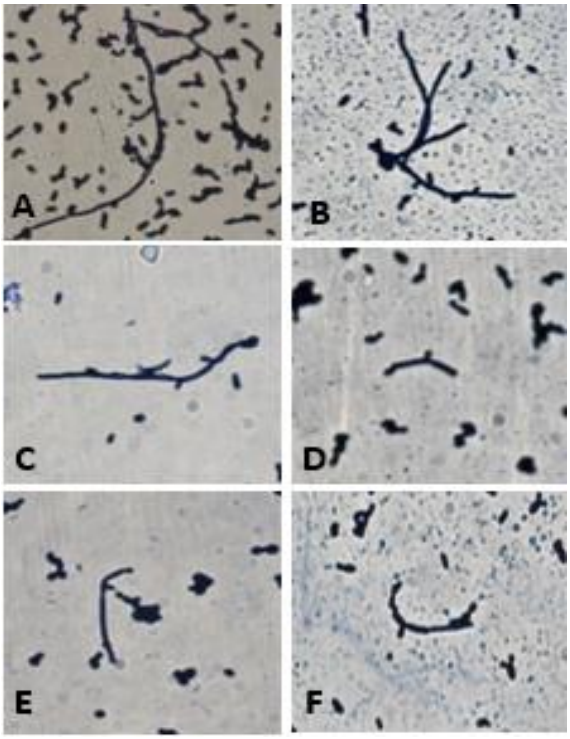

Figure 2. The effect on the dimorphic capacity of *C. albicans*, treated with sub-lethal concentration of the antimicrobial agents. A) Control; B) Cells with AgNPs; C) Cells with ApB; D) Cells with ApB-AgNPs; E) Cells with Flu; and F) Cells with Flu-AgNPs

4. Conclusions

AgNPs alter the antimicrobial capacity of antifungals. Synergistic effect was found in the combined treatment of AgNPs and Amphotericin B, while it was antagonist in AgNPs-Fluconazole. On the other hand, AgNPs, antifungals, neither their combination alter the dimorphic capacity of *C. albicans* under standard culture conditions.

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