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Short communication

Antimicrobial photodynamic therapy on *Candida albicans* pre-treated by fluconazole delayed yeast inactivation

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ABSTRACT

Antimicrobial photodynamic therapy (APDI) has been used to treat localized infection and the aim of this study was to evaluate the effect of APDI combined with fluconazole in suspension of *Candida albicans*.

C. albicans ATCC90028 was subcultured onto Sabouraud agar and inocula were prepared at yeast density of 1×10^6 CFU/mL. Methylene blue (MB) was used with concentration of 100 mM. Yeast cells were incubated for 30 min in 24-well plate and then irradiated by LED (660 nm; 690 mW; A = 2.7 cm^2; I = 250 mW/cm^2) with radiant exposure of 30, 60, and 120 J/cm^2. The same APDI setup was used with 2 h fluconazole (0.5 μg/mL) incubation. A UV–vis optical absorption spectroscopy was achieved following fractionated irradiation up to 960 s.

There were substantial differences in the killing effect following MB-mediated APDI and *C. albicans* was eradicated in the both APDI groups. The fluconazole combined to APDI delayed the complete inactivation of the yeast ($p < 0.05$). Spectroscopy showed a decrease in absorption following irradiation for all absorption peaks.

APDI presented an antagonist effect in the presence of fluconazole.

1. Introduction

Emergence of yeast infection presents a challenge for the medical and scientific communities [1], because opportunistic fungal pathogens may produce localized or invasive infections in immunocompromised and debilitated patients [1–3]. Gonçalves et al. [2] attested to the fact that high level of antifungal drug consumption has increased the number of reported cases of yeast resistance [2]. The therapy for mycoses uses a relative limited number of antifungal drugs, such as fluconazole, itraconazole, and amphotericin-B. The latter is considered the gold standard for severe mycoses treatment despite the risk for producing severe acute and chronic toxicities, such as renal function impairment [3]. A shortage of innovative and effective antifungal drugs exists.

APDI has been used to treat localized infection [4–7] and the photochemical principle of this therapy is the association of light and a photoactive drug [8–10]. As a result of this interaction, a massive amount of reactive oxygen species are formed and it leads to microbial damage and death, including fungal pathogens [10,11]. Snell et al. [12] demonstrated that miconazole can be used to prolong fungistasis in *C. albicans* that survived APDT [12], however ketoconazole and fluconazole did not produced synergistic effect. On the other hand, Quiroga et al. [13] observed an enhancement in the antifungal action by combining fluconazole and APDT. Thus, the aim of this study was to evaluate the effect of APDI combined with fluconazole in suspension of *Candida albicans*.

2. Material and methods

Candida albicans ATCC90028 cells were subcultured from vial stocks under aerobic conditions [9,11]. Fungal inocula were prepared at yeast density of 1×10^6 CFU/mL. The PS was added to the yeast suspension with final concentration of 100 μM [4,8,9,11].

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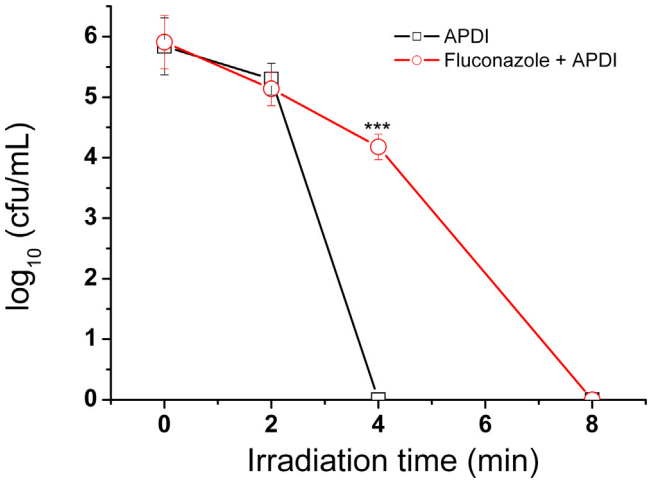

Fig. 1. Yeast inactivation in function of irradiation time on the APDI and APDI + Fluconazole groups. Data (n = 6) represents mean and standard deviation. Statistic significant difference is indicated by symbol (***).

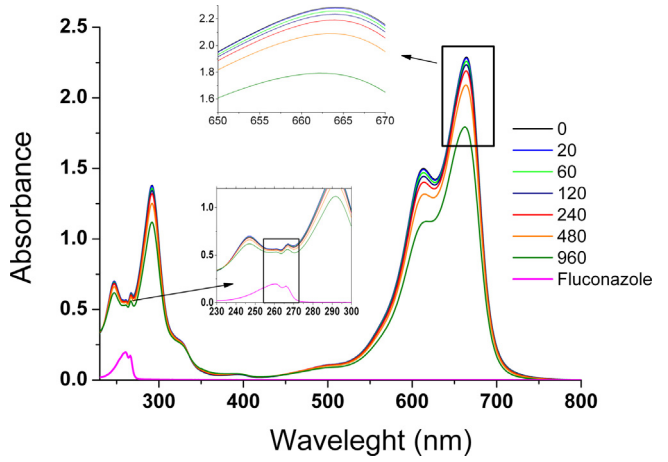

Fig. 2. Optical absorption of MB combined to fluconazole. Absorbance decreased following irradiation at 664, 613, 293, 268, and 247 peaks.

LED (660 nm) with output power of 690 mW was used and the probe was fixed on a holder that kept the beam area at 2.7 cm^2, which coincided to a single well size from the 24-well plate. This parameter provides irradiance of 250 mW/cm^2 in the plaque and radiant exposure of 30, 60, and 120 J/cm^2, correspondent to 2, 4, and 8 min of irradiation. The same setup was used with yeast suspension incubated with fluconazole (0.5 μg/mL) for 2 h [9,12].

C. albicans cultures exposed to APDI were evaluated. For all tests, we used a control group composed of cells incubated with MB but without irradiation. The yeast colonies were counted and converted into CFU/mL for analysis. The control conditions used were suspension without PS and irradiation; suspension with PS in the dark, and suspension irradiated without PS. The experiment was repeated 6 times in triplicate in 3 independent days. One-Way ANOVA was used followed by Bonferroni test. The results were considered statistically different when ($p < 0.05$). Data is presented as the mean and standard deviation of each group.

UV–vis optical absorption spectrometry was performed using a spectrophotometer (CARY 5000, Varian Inc., Santa Clara, CA, USA). Absorption of MB combined to fluconazole (quartz cell; 1 cm path) in distillated water was measured following fractionated irradiation ($\lambda = 660$ nm; $I = 250$ mW/cm^2) at 20, 60, 120, 240, 480, and 960 s.

3. Results

The number of *C. albicans* cells treated only with laser irradiation or with MB alone was similar to the control group in all experiments (data not shown). There were substantial differences in the killing effect following MB-mediated APDI. Fig. 1 demonstrates that *C. albicans* was eradicated in the both APDI groups. The degree of photoinactivation was dependent upon the time of the irradiation and fluconazole combined to APDI delayed the complete inactivation of the yeast. Following two minutes of irradiation no difference was observed between the groups ($p > 0.05$), however, at 4 min of irradiation all cells from APDI group had been inactivated, and only 2 logs of killing was observed on the group of APDI combined to fluconazole ($p < 0.05$). On the end of 8 min of irradiation, both groups presented complete yeast inactivation.

The absorbance spectrum of methylene blue combined to fluconazole showed a decrease in absorption following irradiation (Fig. 2). The fluconazole absorbance presented two peaks at 262 and 267 nm; however, it does not presented absorbance on visible area of electromagnetic spectrum. The methylene blue absorbance presented peaks on UV–vis area.

Following irradiation, the absorbance reduced in function of irradiation time. All peaks of absorption presented the same behave with a decrease of about 20% after 960 s of irradiation. The 664 nm peak presented absorbance of 2.243 before irradiation it reach 1.784 after the complete irradiation time.

4. Conclusion

In conclusion, APDI presented an antagonist effect in the presence of fluconazole and it delayed yeast inactivation. In addition, the absorbance of fluconazole/MB solution decreased with light irradiation.

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