

Fungicidal effect of methylene blue photoactivated on *Cryptococcus neoformans*. *In vitro* and *in vivo* studies

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Background: Cryptococcosis is an infection caused by the encapsulated yeast *Cryptococcus neoformans* and the most afflicted sites are lung, skin and central nervous system [1]. Countless studies reported that photodynamic therapy (PDT) can inactivate yeast cells[2-4]; however, the *in vivo* experimental models of cryptococcosis photoinactivation are not commonly described. The aim of this study was to investigate the ability of methylene blue (MB) combined with a low-power red laser to inactivate *Cryptococcus neoformans in vitro* and *in vivo* experimental models, as well as observe morphological changes on cells following PDT.

Material and Methods: To perform the *in vitro* study, suspension of *C. neoformans* ATCC-90112 (10^6 cfu/mL) was used. The light source was a laser (Photon Lase III, DMC, São Carlos, Brazil) emitting at $\lambda=660$ nm with output power turned to 30 and 90 mW resulting fluence rates of 100 and 300 mW/cm² at 3, 6, and 9 minutes of irradiation[2]. One hundred- μ M MB was used as photosensitizer[2]. Yeast cells were viewed with scanning electron microscope (Phillips XI, Eindhoven, Holland). Sets of cultures from control group and PDT group were prepared. For the *in vivo* study, 10 BALB/c mice had the left paw inoculated with *C. neoformans* (10^6 cfu). Twenty-four hours after inoculation, PDT was performed using 150 μ M MB and 100mW red laser with fluence at 180J/cm².

Results: The reduction of yeast cells viability in the tested PDT groups was effective in the used parameters. The overall finding was that MB-stained yeast cells are killed in a light fluence and fluence rate dependent manner *in vitro* after red illumination and the fungicidal effect was dependent on the exposure time. The fluence rate of 100mW/cm² presented a moderate fungicidal effect. On the other hand, the reduction of yeast cells was more effective in the fluence rate of 300mW/cm². In addition, electron scanning microscopy examination of cells in control group revealed typical globular cells. Conversely, the yeast cells harvested from PDT group presented structural morphological changes compared to the control group. It was observed that yeast cells languish and their capsule are not so smooth (figure 1).

In the *in vivo* experiment, photodynamic effect was able to kill yeast cells in the mice infected paw following red laser irradiation. Animals treated by PDT presented 1 log reduction of *C. neoformans* viability ($p<0.01$).

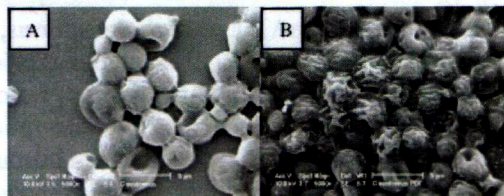


Fig.1 – Scanning electron microscopy of *C. neoformans* yeast cells are present in the figure (A) without treatment, and (B) following PDT. The yeast cells shown are representative of those seen for each condition. Scale bars represent 5 μ m.

Conclusion: Photodynamic therapy presented effect to treat dermal cryptococcosis; however, more effective parameters must be found for *in vivo* studies.

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