TITLE: CALCIUM CHANNEL BLOCKERS AS POTENTIAL ANTIFUNGAL DRUGS AGAINST *CRYPTOCOCCUS NEOFORMANS*

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ABSTRACT:
Cryptococcus neoformans is the etiological agent of cryptococcal meningoencephalitis, an important opportunistic infection in immunocompromised individuals that corresponds to the third most common neurological complication in AIDS patients. The recommended treatment with amphotericin B and fluconazole is 50% to 80% effective, with toxicity and resistance as recurrent problems. This indicates the importance of searching for new targets and drugs for cryptococcosis treatment. Re-purposing drugs already used in the clinic that demonstrate antifungal activity have proved promising. Considering the importance of calcium storage mediated by calcium transporters on cryptococcal virulence, the aim of this study was to evaluate the use of calcium channel blocker drugs as an alternative therapy for cryptococcosis. The minimum inhibitory concentration (MIC) of amiodarone was determined by broth microdilution test for H99 strain (wild-type) and null mutant strains for calcium transporters Pmc1 (*pmc1*) and Vcx1 (*vcx1*). The H99 strain presented amiodarone MIC of 12,5 µg/mL, while *pmc1, vcx1* and *pmc1vcx1* mutant strains presented a MIC of 6,25 µg/mL. The synergy between amiodarone and fluconazole was evaluated by the checkerboard method. The FIC index (0,75) observed for H99 and null mutant strains indicates synergy between the drugs. The in vivo antifungal effect of amiodarone in combination with fluconazole is under analysis using a mice model of cryptococcosis. Two important *C. neoformans* virulence factors, capsule formation and melanin production, were evaluated in the presence of 3,12 µg/mL of amiodarone. The relative capsule size of H99 was reduced by amiodarone exposure. Also, we observed that amiodarone influences the secreted melanin of H99 and *pmc1* cells within 24 hours. The relative expression of important genes involved in the calcium-calcineurin signaling pathway were evaluated by RT-qPCR. The transcripts of *CRZ1* and *VCX1* were upregulated by amiodarone exposure in H99 and *pmc1* strains, respectively. These data indicate a potential use of amiodarone in combination with fluconazole as a new possibility for cryptococcosis management.

Keywords: *Cryptococcus neoformans*, amiodarone, calcium, fluconazole, synergy

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