

Título Heteroresistance to itraconazole increases the virulence of *Cryptococcus gattii*

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Long-term maintenance therapy with azoles creates favorable conditions for the emergence of resistance to the drug. There are evidences that clinical recurrences of cryptococcal meningitis result from persistence of the original infecting strain, despite azole therapy. Heteroresistance is an adaptive mechanism employed by the microorganism to counteract the stress of increasing drug concentration, and it may enhance the fitness to survive under itraconazole stresses as well. Itraconazole is one of the azoles used in the therapy of cryptococcosis. In this study, we evaluated the heteroresistance of *Cryptococcus gattii* to itraconazole and their relationship with virulence. The effects of the heteroresistance to itraconazole were studied in L135/03 strain of *C. gattii* by performing: survive curve, bronchoalveolar lavage fluid (BALF) analysis, colony forming units and myeloperoxidase activity (MPO). Mice infected by an intratracheal route with heteroresistant clones from *C. gattii* strain L135/03 succumbed significantly earlier ($p = 0.04$) than those infected with original cells. After 10 days postinoculation with yeast cells, the fungal burden in lungs and BALF were significantly higher in mice infected with the heteroresistant cells. In fact, the original L135/03 strain did not disseminate to the brain until 10 days post-inoculation, while the heteroresistant cells colonized the brain. Mononuclear leukocytes and neutrophils were more recruited to the lungs by the heteroresistant cells than the original strain ($p < 0.05$). The MPO activity in the lungs confirmed higher neutrophil influx with the heteroresistant yeasts ($p < 0.05$). Our results indicate that heteroresistance to itraconazole increases the virulence of *C. gattii*. This phenomenon may represent an additional mechanism of drug action that could contribute to relapses of cryptococcosis during itraconazole therapy.

Palavras-Chaves *Cryptococcusgattii*, heterorresistência, itraconazol

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