Title: ALTERATION OF *Cryptococcus gattii* VIRULENCE FACTORS DURING INFECTION INTRATRACHEAL IN MURINE MODEL

Authors Fontes, A.C.L. [1], Oliveira, L.V.N. [1], Barcellos, V.A. [2], Vainstein, M.H. [2], Santos, D.A. [1]

Institute [1] Departamento de Microbiologia - Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brasil (Av. Antônio Carlos, 6627, Pampulha - Belo Horizonte - MG - CEP 31270-901), [2] Centro de Biotecnologia - Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brasil (Av. Bento Gonçalves, 9500, Campus do Vale - Porto Alegre - RS - CEP 91501-970)

Abstract:

Cryptococcosis caused by *Cryptococcus gattii* is a systemic infection can be shown as an asymptomatic pulmonary colonization, even how meningitis or disseminated disease. *C. gattii* usually cause disease in immunocompetent hosts. The polysaccharide capsule is important for the survival of the micro-organism in the environment and in the host and is considered an important virulence factor. The availability of antifungal therapy for this disease is low and provides certain toxicity, at the same time the incidence of infections caused by resistant strains increased. Thus, the aim of this study was to evaluate the virulence factors of *C. gattii* after infection in mice treated with fluconazole (FLC). The project was approved by the Ethics Commission on the use of animals UFMG. The L27/01 strain was used at a concentration of 1 x 106 CFU/animal to intratracheal infection of C57BL mice. They were separated in two groups: treated and not treated with 10mg/kg/day of FLC. After 15 days of infection, and 10 days of treatment with FLC, lungs and brains were macerated and plated in Petri dishes with YPD medium to obtain a CFU per gram of tissue and the following virulence factors: cell growth, melanization, production laccase and size of polysaccharide capsule. The count of CFU/g of organ showed a greater fungal load in the lung than in the brain independent of the sample group. There was no significant difference in growth the colonies at 30 °C and 37 °C of both groups. Melanin production was significantly higher in the treated group recovered colonial confirmed by production of laccase in the treated group exhibited OD (optical density) of 0.48 while the untreated group showed OD of 0.11 ($p < 0.001$). Capsule size of the treated group showed a significant difference, with an average of 3.89 µm while the untreated group was 1.96 µm ($p < 0.05$). The results show that treatment with FLC alter the virulence factors of *C. gattii,* even after 10 days of treatment. Knowledge of way in which fluconazole resistance factors affect the virulence of *C. gattii* is essential to understand the clinical impact of antifungal resistance in cryptococcosis. Due to the increasing number of cases and deaths by cryptococcosis, studies of *C. gattii* and factors addressing the antifungal resistance should be a research priority.

Keywords: *Cryptococcus gattii*, fluconazole, virulence factors, polysaccharide capsule, melanization

Financial support: CNPq, CAPES and FAPEMIG