Effect of fluconazole treatment on the profile of CD11b/Ly-6G neutrophils migrating to the site of Paracoccidioides brasiliensis infection

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Introduction: Paracoccidioidomycosis (PCM) is a systemic mycosis caused by the *Paracoccidioides* spp. fungal complex. It presents slow progression and complex treatment, justifying studies that broaden the understanding of the immune response mechanisms that induce a protective response against fungal infection. New therapeutic approaches, aiming to improve the performance of the immune response against the fungus, seek to understand the action of antifungals, mainly on neutrophils (PMN), which play a fundamental role as the first line of defense against infection. **Objective:** The central objective of this study is to evaluate the use of the antifungal drug Fluconazole in experimental PCM treatment and verify if this medication alters the immunological pattern developed during the disease, acting in the control of inflammation caused by the fungus and, simultaneously, exerting antifungal action by eliminating viable fungi at the site of infection. **Methodology:** A subcutaneous animal model was used to evaluate the influx of neutrophils (PMN) to the infection site. Infection was performed with $1x10^6$ cells/mL of *P. brasiliensis* (virulent Pb18 isolate) suspension via air pouch. The animals were treated with fluconazole subcutaneously directly into the air pouch during the last 3 days of infection, and after 10 days of infection, the cell exudate, rich in neutrophils was collected. The obtained cells were blocked with mouse anti-CD16/CD32 monoclonal antibodies (Fc-Block) and stained for surface markers using APC-labeled mouse anti-Ly- 6G and FITC-labeled mouse anti-CD11b antibodies (BD Biosciences, San Jose, CA, USA). Fluorescence acquisition was performed on a FACS Lyric™ flow cytometer (BD Biosciences, San Jose, CA, USA) and the data were analyzed using FlowJo software (BD Biosciences, San Jose, CA, USA), and analyzed using BD FACSuite™ software. **Results:** Flow cytometry analyses for neutrophil labeling showed that the group treated with Fluconazole had a significantly higher proportion of neutrophils compared to the control group (only Pb18). Furthermore, the neutrophils in the Fluconazole-treated group showed a different profile than the control group, with an increase in double-labeled neutrophils, indicating that this population has a higher number of active neutrophils and no other
phagocytic cells types in contrast to the control group. **Conclusion:** The data show that the neutrophil population in the group treated with Fluconazole has a larger size, which may indicate an increase in phagocytic activity at the site of infection. As these cells are the first line of defense against Pb18, this change in immune response profile suggests that Fluconazole not only acts as an antifungal but also improves the primary immune response against the virulent strain of the fungus, enhancing PMN response.

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