TITLE: INFLUENCE OF LMM11 IN PHAGOCYTOSIS AND KILLING OF *Candida krusei*

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ABSTRACT:

By *in silico* methods a novel compound (LMM11) was identified against thioredoxin reductase (TrxR) from *Candida albicans*. This compound showed antifungal activity *in vitro* and *in vivo* against *C. krusei*. It is known that phagocytosis is an important role in the host defense against *Candida* infections and essential for an effective antifungal response. However, many pathogens have developed pathways to evade killing once they have been phagocytosed. Thus, the aim of this study was to evaluate the influence of LMM11 compound on the phagocytosis and killing of *C. krusei* by macrophages. *C. krusei* ATCC 6258 treated (CkT+MØ) or not (Ck+MØ) with minimum inhibitory concentration (MIC) of LMM11 compound were added to 24-well plates containing sterile glass coverslip with a pre-adhered monolayer of J774-A1 macrophages (5:1) and incubated for 2 hrs/37°C/5% CO_2. Then, the glass coverslips were washed and stained with haematological rapid staining and evaluated in 100 cells: the phagocytic index (PI) (average of internalized yeasts X percentage of phagocytic macrophages). Parallel to phagocytosis, killing capacity of the macrophages was evaluated. Yeasts were incubated with macrophages under the same conditions. After 2hrs of incubation, non-phagocytosed yeasts were removed and the macrophages were lysed with sterile ice water. Lysate dilutions were plated on SDA plates and incubated for 24h for determination of CFU/mL. There were no significant differences in the percentage of phagocytic macrophages in the two groups analyzed: Ck+MØ (45.5% ± 6.4) and CkT+MØ (40% ± 2.8). The mean of internalized yeasts was similar between Ck+MØ (5.3 ± 0.42) and CkT+MØ (5.4 ± 0.11). The phagocytic index remained without significant differences for Ck+MØ (241.5 ± 29) and CkT+MØ (215.4 ± 0.61). The killing capacity of the macrophages was evaluated by the reduction Log_{10} CFU/mL. The group treated with the LMM11 compound (CkT+MØ) showed a reduction statistically significant ($p<0.05$) of the CFU in relation to the control (Ck+MØ). LMM11 compound did not influence the percentage of phagocytic macrophages, phagocytic index and mean of phagocytosed yeasts. However, the killing capacity of the group treated with compound LMM11 showed a reduction of the CFU/mL in relation to control. Thus, considering that LMM11 acts on thioredoxin system, we believe that inhibition of this system induces a reduction of the detoxifying capacity of yeasts and making them more susceptible to death by the macrophages.

Keywords: *Candida krusei,* LMM11 compound, phagocytosis.

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