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1er Premio

Usefulness of *Galleria mellonella* invertebrate model for studying the *Cryptococcus neoformans* host-pathogen-antifungal interactions

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Background:

Galleria mellonella larva (GML) is an invertebrate model suitable for researching the host-pathogen interactions of several microorganisms. This infection-model offers a useful tool for studying the virulence and *in vivo* antifungal efficacy against *Cryptococcus neoformans* (*Cn*) clinical isolates.

Objective:

1-To evaluate the virulence of *Cn* clinical isolates in GML model; 2-To evaluate the antifungal efficacy in this infection-model.

Method:

Two *Cn* clinical strains, isolated from patients who developed AIDS-associated cryptococcal meningitis with failure of antifungal treatment, were included.

Antifungal drugs: amphotericin B (AB) (1 ug/gr/larva), voriconazole (VZ) (1 ug/gr/larva) and fluconazole (FZ) (20 ug/gr/larva) were tested.

Inoculum: 10 uL of 10⁶ cel/mL of *Cn* was inoculated in the last left pro-leg of each GML in all experiments. Sixty GML per plate were placed on 10 sterile Petri plates: 1-GML control without inoculation; 2-GML inoculated with PBS; 3-GML inoculated with *Cn*; 4 to 9-GML post *Cn* inoculation were treated at time 0 and 24 h, at 28°C and 37°C, with 10uL of AB, FZ and VZ respectively; 10-GML + *Cn* + 10 uL of Calcofluor white. Separately, the antifungal drugs were inoculated into GML, without *Cn* strain, to prove if the drugs are toxic by themselves. Plates were incubated at 28°C and 37°C. Reading was carried out every 24 h during 15 days. The assays were developed in duplicate. Percentage of death was calculated.

Results

During GML infection, the cell and capsule size of *Cn*, and the haemocytes count, were all increased considerably compared with *Cn* strains and GML before inoculation ($p < 0.001$). Overall, 50% of larvae death without treatment was at 48 h and 72 h post *Cn* inoculation at 28°C and 37°C respectively. The protective antifungal activity was observed in the group treated with antifungal drugs at 24 h post *Cn* inoculation. For AB, VZ and FZ, larval survival was at 7, 14 and 15 days, respectively. No significant differences were observed among groups incubated at 28°C or 37°C.

Conclusions: GML model seems to be an attractive tool to study the host-pathogen-antifungal interactions. The protective activity of antifungal drugs depends on the challenge time and the drug used.