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Paradoxical effect to caspofungin in *Candida* species does not confer survival advantage in a *Drosophila* model of candidiasis

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The paradoxical growth (PG) to echinocandins has been observed in vitro in *Candida* and *Aspergillus* species.[1] Its mechanisms are incompletely understood, although activation of calcineurin and chitin biosynthesis pathways have been implicated.[2-4] Importantly, the in vivo relevance of PG is not well defined as there is paucity of both animal model and clinical data on the implications of this phenomenon.[5]

As PG in vivo has been studied in the context of echinocandin exposure, it remains unknown whether there are differences in virulence of *Candida* strains showing or not showing PG when they infect the host in the absence of echinocandin pressure. To that end, we examined differences in virulence of *Candida* isolates showing or not PG in our Toll-deficient fly model of candidiasis, a model we have shown good concordance with mouse models of candidiasis.[6] We used 20 clinical bloodstream isolates: 10 *Candida albicans* (5 with PG to caspofungin, 5 without PG) and 10 *C. tropicalis* (5 with PG, 5 without PG).[1] All isolates had comparable growth in vitro in the absence of caspofungin (data not shown). We used female Oregon^R Toll-deficient flies and followed standard procedures for feeding, housing and manipulation of flies.[6] The flies were exposed to a 12-h light/dark cycle. All *Candida* strains were grown on yeast-peptone-dextrose (YPD) agar medium. For the infection experiments, cultures of each strain were grown overnight at 30 °C on YPD liquid medium before collection of yeast-cells, which were counted using a hemocytometer and then suspended in sterile phosphate buffered saline. The injection method was used for fly infection. Specifically, we injected flies (n = 23 per experimental group) in the thorax with a 0.1 μm thin needle that had previously been dipped in a concentrated solution of 5×10^5 *Candida* yeast cells/ml (~1×10^2 yeast cells per fly), as previously described.[6] After infection, the flies were maintained at 29 °C and were transferred to fresh vials every 2 d. Survival was assessed daily up until day 7 after infection. Flies that died within 3 h post-injection were excluded from the survival analysis. Each experiment was performed in triplicate on different days. Survival curves were plotted using Kaplan-Meier analysis and differences in survival rates were analyzed using the log-rank test in the GraphPad Prism software (version 5.0; GraphPad Software, Inc.). A P value of ≤0.05 was considered statistically significant.

We observed no differences in fly mortality between *C. albicans* and *C. tropicalis* isolates irrespective of PG (**Fig. 1A and B**). As hyperacute infection caused by a high inoculum of fungi could mask subtle differences in strain virulence, we repeated the experiments using a 10-fold lower inoculum, but, again, no differences in survival were detected between strains showing or not PG (data not shown). Next, as PG seems to be modulated by the calcineurin pathway,[2-4] and stimulation of chitin synthesis may represent a rescue mechanism against caspofungin activity,[3] we asked whether pre-exposure of *Candida* to the calcineurin inhibitor cyclosporine or to a sub-inhibitory caspofungin concentration uncovers subtle differences in virulence among *Candida* strains showing or not PG. To that end, we inoculated *C. albicans* and *C. tropicalis* (one strain each) showing PG onto YPD liquid medium with or without cyclosporine (1 μg/ml) (**Fig. 1C and D**) and with or without caspofungin (MIC/2) (**Fig. 1E and F**), and incubated overnight at 30 °C. Thereafter, *Candida* yeast cells were washed three times with PBS and injected onto *Toll*-deficient flies. Survival was assessed as previously described. Again, no mortality differences were appreciated. In conclusion, using multiple, yet genetically unmatched *C. albicans* and *C. tropicalis* strains having comparable growth rates in a defined mini-host model of experimental candidiasis, our data imply that PG to caspofungin is unlikely to be linked to virulence differences, even in the presence of echinocandin pre-exposure.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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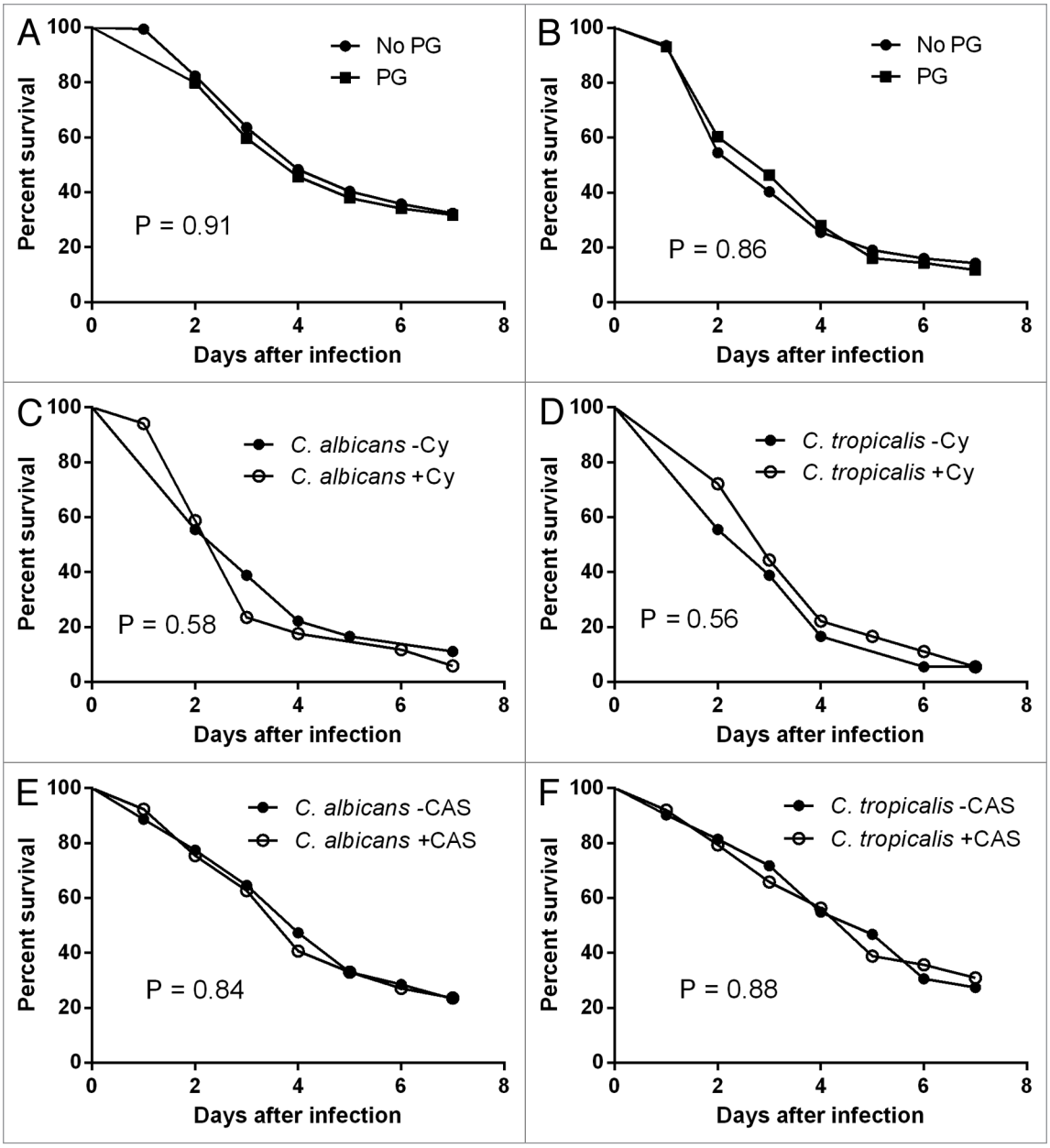

Figure 1. Survival rates of Toll mutant flies injected with a needle previously dipped in a solution containing 5×10^5 *C. albicans* (**A**) and *C. tropicalis* (**B**) yeast cells/ml each showing ($n = 5$) or not showing ($n = 5$) paradoxical growth (PG) against caspofungin. Survival rates of Toll mutant flies injected with a needle previously dipped in a solution containing 5×10^5 yeast cells/ml which were grown overnight at 30 °C in the presence or not of 1 μg/ml cyclosporine (**C and D**) and in the presence or not of caspofungin (MIC/2) (**E and F**). Data shown are the means of 3 independent experiments ($n = 23$ flies/group).

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