Adaptation mechanisms to amphotericin B and echinocandins in *Candida* species

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Current therapy against fungal infections is based on the recovery of the patient immune system and on the administration of antifungal drugs, which are mainly polyenes (amphotericin B), azoles (fluconazole, voriconazole, itraconazole and posacazonale) and echinocandins (caspofungin, anidulafungin and micafungin). Amphotericin B acts through pore formation after binding to ergosterol. Azoles block ergosterol synthesis by inhibiting the 14-α-sterol demethylase. Echinocandins affect cell wall formation by inhibiting the enzyme β-1,3-glucan synthase encoded by the FKSs gene. In the laboratory, we are interested in the effects of these antifungals on yeasts and on the resistance mechanisms. We are focusing our studies on amphotericin B and caspofungin. We have observed that Amphotericin induces a strong oxidative burst in a wide variety of fungi, which occurs previous to the death of the cells. One of our main goals is to elucidate if the induction of oxidative damage is necessary for the antifungal effect of the molecule, and for this purpose we are characterizing the effect of AmB on resistant strains. Our preliminary results indicate that resistance to AmB is associated with reduction in the oxidative damage and induction of antioxidant enzymes, such as catalase and superoxide dismutase. These findings indicate that AmB exerts multiple effects in the cell and that pore formation is not sufficient to induce yeast death.

Concerning echinocandins, we have focused our studies in the characterization of the “paradoxical (or Eagle) effect”. This phenomenon happens when the yeast cells are susceptible to intermediate conditions of the antifungal, but become tolerant at high concentrations. We have demonstrated that this effect is not due to an inactivation of the drug at high concentrations, and truly reflects an adaptation mechanism of the cells to the antifungal. Growth under paradoxical conditions is associated with lack of hyphae formation, cell enlargement and chitin accumulation. Moreover, virulence of cells grown in the presence of high caspofungin concentrations was attenuated in the non mammalian model *Galleria mellonella*. Overall, these findings suggest that high caspofungin

concentrations induce an adaptative response that results in cell wall rearrangement and tolerance to the antifungal, but this response is not sufficient to produce disease in the host.

In summary, our findings indicate that the adaptation to antifungals is a complex process that affects not only the tolerance to the drug, but also the virulence of the yeast. The full characterization of antifungal action mechanisms and of the pathways involved in resistance and tolerance has important clinical consequences because they will contribute to improve the current available therapies.