Note

Antifungal susceptibility of *Saccharomyces cerevisiae* and therapy in a murine model of disseminated infection

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**A B S T R A C T**

**Background:** The incidence of systemic infections by *Saccharomyces cerevisiae* has increased in recent years, especially among immunocompromised patients. Amphotericin B, voriconazole or echinocandins have been used with favorable outcome against systemic infections by this fungus. However, clinical experience is limited and no in vivo studies have been conducted.

**Aims:** We evaluated the in vitro activity of nine antifungal compounds against *S. cerevisiae* and the in vivo efficacy of those three antifungals showing the highest in vitro activity by using a murine model of systemic infection.

**Methods:** Minimal inhibitory concentrations (MICs) were determined by the microdilution method against three strains of *S. cerevisiae*. After intravenous infection with $5 \times 10^7$ CFUs, animals received liposomal amphotericin B (5 mg/kg), voriconazole (25 mg/kg) or anidulafungin (5 mg/kg). Treatment efficacy was assessed by determining of CFUs/g in liver, kidney, brain, lung and spleen.

**Results:** 5-Fluorocytosine was the most in vitro active compound followed by amphotericin B, voriconazole and anidulafungin. The in vivo study showed that liposomal amphotericin B was the most effective drug driving highest fungal clearance.

**Conclusions:** All treatments reduced the fungal load in comparison to the control group, being liposomal amphotericin B the most effective drug followed by anidulafungin and finally voriconazole.

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**Sensibilidad antifúngica de *Saccharomyces cerevisiae* en un modelo murino de infección diseminada**

**R E S U M E N**

**Antecedentes:** La incidencia de infecciones sistémicas causadas por *Saccharomyces cerevisiae* ha aumentado en los últimos años, especialmente entre pacientes inmunodeprimidos. A pesar de que la anfotericina B, el voriconazol o las equinocandinas han dado buen resultado en infecciones sistémicas por este hongo, no se han establecido recomendaciones terapéuticas sólidas.

**Objetivos:** Se evaluó la actividad in vitro de nueve antifúngicos frente a *S. cerevisiae* y la eficacia in vivo de los tres fármacos con mayor actividad in vitro mediante un modelo murino de infección sistémica.

**Métodos:** Se determinaron las concentraciones mínimas inhibitorias (CMIs) frente a tres cepas de *S. cerevisiae* por el método de microdilución. Después de la inocular intravenosa con $5 \times 10^7$ UFC, los ratones fueron tratados con anfotericina B liposomal (5 mg/kg), voriconazol (25 mg/kg) o anidulafungina (5 mg/kg). La eficacia de los tratamientos se estableció basándose en la determinación de UFC/g en hígado, riñón, cerebro, pulmón y bazo.

**Resultados:** La 5-fluorocitosina fue el compuesto más activo in vitro, seguido por la anfotericina B liposomal, el voriconazol y la anidulafungina. En el estudio in vivo, la anfotericina B liposomal fue el fármaco más eficaz en términos de reducción de la carga fúngica y esterilización de los órganos estudiados.

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Saccharomyces cerevisiae es un hongo distribuido de forma amplia, comúnmente usado en la producción de alimentos, bebidas alcohólicas y diferentes procesos biotecnológicos.\(^1\)\(^,\)\(^2\) A pesar de su uso beneficioso, S. cerevisiae también puede actuar como un patógeno oportunista causando una variedad de infecciones en individuos inmunocomprometidos, como infecciones del tracto respiratorio, genitourinario, peritonitis, abscesos y fístulas rectovaginales, entre otras.\(^3\)\(^,\)\(^4\)\(^,\)\(^5\)\(^,\)\(^6\)\(^,\)\(^7\)\(^,\)\(^8\)\(^,\)\(^9\)\(^,\)\(^10\)\(^,\)\(^11\)\(^,\)\(^12\)\(^,\)\(^13\)\(^,\)\(^14\)\(^,\)\(^15\)\(^,\)\(^16\)\(^,\)\(^17\)\(^,\)\(^18\)\(^,\)\(^19\)\(^,\)\(^20\)\(^,\)\(^21\)\(^,\)\(^22\)\(^,\)\(^23\)

El tratamiento recomendado para estas infecciones generalmente incluye amfotericina B (AMB) o AMB liposomal, y 5-fluorocitosina (5FC) en los casos más graves, pero pocos estudios han evaluado la eficacia de otros fármacos antifúngicos. En un estudio anterior, se demostró que S. cerevisiae puede actuar como un patólogo asociado. La resistencia a los fármacos antifúngicos se ha observado en estudios de varios casos.\(^3\)\(^,\)\(^4\)\(^,\)\(^5\)\(^,\)\(^6\)\(^,\)\(^7\)\(^,\)\(^8\)\(^,\)\(^9\)\(^,\)\(^10\)\(^,\)\(^11\)\(^,\)\(^12\)\(^,\)\(^13\)\(^,\)\(^14\)\(^,\)\(^15\)\(^,\)\(^16\)\(^,\)\(^17\)\(^,\)\(^18\)\(^,\)\(^19\)\(^,\)\(^20\)\(^,\)\(^21\)\(^,\)\(^22\)\(^,\)\(^23\)

La adquisición de infecciones por S. cerevisiae ha sido recientemente relacionada con el uso de probióticos o suplementos dietéticos, algunos de los cuales podrían servir como un recurso más efectivo para el manejo de este organismo.\(^1\)\(^,\)\(^2\)\(^,\)\(^3\)\(^,\)\(^4\)\(^,\)\(^5\)\(^,\)\(^6\)\(^,\)\(^7\)\(^,\)\(^8\)\(^,\)\(^9\)\(^,\)\(^10\)\(^,\)\(^11\)\(^,\)\(^12\)\(^,\)\(^13\)\(^,\)\(^14\)\(^,\)\(^15\)\(^,\)\(^16\)\(^,\)\(^17\)\(^,\)\(^18\)\(^,\)\(^19\)\(^,\)\(^20\)\(^,\)\(^21\)\(^,\)\(^22\)\(^,\)\(^23\)

En la presente investigación se evaluó la actividad in vitro de AMB, FLC, PSC, VRC, anidulafungina (AFG), SFC, itraconazol (ITC), caspofungina (CFG) y micafungina (MFG) contra S. cerevisiae y se determinaron los parámetros de la cinética de tiempo de muerte celular como así como la eficacia in vivo de los compuestos más activos.

Fig. 1. Cinética de muerte celular de a) AMB, b) VRC y c) AFG contra tres cepas de S. cerevisiae ( ■ ) 0.06 µg/ml, ( □ ) 0.125 µg/ml, ( △ ) 0.5 µg/ml, ( ▪ ) 2 µg/ml, ( ○ ) 8 µg/ml, ( ● ) 32 µg/ml, ( ■ ) control.
For each strain and drug assayed, 8 mice were included. Mice were infected intravenously (i.v.) via the lateral tail vein with $5 \times 10^7$ CFU in 0.2 ml of 0.9% saline. Therapies consisted of liposomal AMB (LAMB) (AmBisome; Gilead Sciences S.A., Madrid, Spain) administered i.v. at 5 mg/kg once daily (QD), VRC (Vfend, Pfizer S.A., Madrid, Spain) at 25 mg/kg given orally by gavage (p.o.) QD or AFG (Ecalta, Pfizer S.A.) at 5 mg/kg intraperitoneally (i.p.) QD. In vivo assayed drugs were chosen according to the in vivo results obtained, and doses were selected based on previous studies.\(^5,27\) From 3 days before infection, mice treated with VRC received grapefruit juice instead of water, as grapefruit juice is an inhibitor of cytochrome P450 enzymes, which display an extensive metabolism in mice resulting in elevated drug clearance.\(^5\) Control groups received no treatment. Drug efficacy was evaluated according to the fungal burden reduction in brain, liver, spleen, lungs and kidneys. Despite the high in vitro activity of 5FC, this drug was not included into the in vivo study due to its known toxicity.\(^19\) At day 8 post-infection animals were euthanized by CO\(_2\) inhalation and organs were aseptically removed, homogenized in 1 ml of sterile saline, ten-fold diluted and placed onto PDA for CFU/g determination. Results from the tissue burden studies were analyzed using the Mann–Whitney U-test by Graph-Pad Prism 6.0 for Windows (GraphPad Software, San Diego California USA). A p value of $\leq 0.05$ was considered statistically significant.

Despite FLC has been used in combination with AMB to treat systemic infections caused by *Saccharomyces*, this drug showed no in vitro activity (MIC $\geq 32 \mu g/ml$) against the strains we tested. This has also been reported by others.\(^7,20\) The rest of the assayed
antifungals showed greater activity (MIC ≤ 1 μg/ml), being 5FC the most active compound (MIC ≤ 0.03 μg/ml) (Table 1). In the time-killing assay, VRC displayed fungicidal activity while AMB and AFG showed fungicidal effect in a concentration dependent manner starting at a drug concentration of 0.06 μg/ml at 8 h in both cases (Fig. 1).

In the in vivo study, control animals showed high fungal load in all studied organs (ranging from 10^5 to 10^6 CFU/g tissue) with the exception of the spleen, in which fungal load was slightly lower (10^7–10^8 CFU/g) (Fig. 2). All assayed treatments were significantly effective in burden reduction from all organs in comparison with the control group regardless of the infecting strain (p < 0.0043). AMB, which is the recommended drug against Saccharomyces infections, showed fungicidal activity correlating with our in vivo results, as it displayed the highest efficacy against all strains with great clearance effect in its liposomal formulation, LAMB (Fig. 2). LAMB was especially effective against strains FMR 13211 and FMR 13213, for which fungicidal effect was observed at MIC concentration, resulting in undetectable CFUs from lung, kidney and spleen and in significant CFU reduction from liver and brain in comparison to VRC or AFG treatments (p = 0.0022). The obtained results with LAMB sustain clinical reports, which although scarce, have shown efficacy of AMB-based therapies. In our case, AMB was the second most effective therapy corroborating previous observations of echinocandins efficacy against Saccharomyces in the clinical settings. Both drugs, LAMB and AFG exhibited similar efficacy against all assayed strains independently of the MIC and the kill-curves obtained. Finally, as previously reported, VRC displayed good activity although it showed lower efficacy than LAMB or AFG treatments.

Guidelines for infections by S. cerevisiae recommend the use of LAMB and echinocandins, as well as the discontinuation of S. cerevisiae as probiotic, especially in vulnerable populations. Although a few strains have been tested, our study contributes with new evidence to prove LAMB treatment effectiveness against experimental invasive infection by S. cerevisiae, as well as the potential use of AFG (and in lesser extent, VRC) as alternative treatments for disseminated infections caused by this fungus.

## References


