Ca37 monoclonal antibody inhibits *Candida albicans* growth *in vitro* and *in vivo*

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INTRODUCTION

Candida albicans is an opportunistic fungus, but it can cause severe infections in patients with impaired immune system. It is the first fungal agent related to health care-associated infections and the fourth causative agent of bloodstream infections. Despite the use of antifungals, mortality rates are still high (50%). Moreover, the use of antifungals leads to toxicity and resistance to the most used drugs are emerging. Therefore, there is an urgent need to find alternative treatment options. In this sense, monoclonal antibodies (mAbs) have shown to play a protective role in animal models.

OBJECTIVE

The aim of this work was to analyze and characterize the effect of a monoclonal antibody, Ca37, directed against a metabolic protein of *C. albicans*

RESULTS

1. Effect of Ca37 mAb on *C. albicans* growth curves

Two strains of the fungus, NCPF 3153 and CECT 13062, were incubated in Sabouraud broth in presence of two Ca37 mAb concentrations. The growth of the reference strain, NCPF 3153, was reduced 30% in presence of 10 µg/ml of the mAb. However, the growth of CECT 13062 was not affected.

Figure 1. Growth curves of two *C. albicans* strains in presence of the mAb. (A) NCPF 3153 and (B) CECT 13062. The fungal strains were incubated with the mAb at 37°C and growth was measured for 24 h at 600 nm of absorbance. The obtained absorbance values were transform into cell densities. Mean values of the experimental replicates are shown.

2. Inhibition of *C. albicans* growth in presence of Ca37 mAb

Candida albicans was incubated with different concentrations of the Ca37 mAb, and 10 µg/ml was the one inducing the highest inhibition of growth (data not shown).

Then, six *C. albicans* strains were incubated with this concentration and, except for the fluconazole-resistant strain UPV 93-153, the growth of all of them was reduced between 70% to 90%.

Figure 2. Growth inhibition of six *C. albicans* strains in presence of 10 µg/ml of the mAb. Yeasts cells were incubated with the mAb for 18 h at 37°C, and then plated for CFU counting. Statistically significant differences in comparison to the non-treated control, *p < 0.05, **p ≤ 0.01, ***p ≤ 0.001.

3. Reduced MIC values in combination with antifungal drugs

Ca37 (µg/ml)	Amphotericin B (mg/L)									
	16	8	4	2	1	0.5	0.25	0.125	0.06	0.03
0	0.142	0.102	0.103	0.106	0.107	0.106	0.106	0.104	1.275	1.015
20	0.111	0.109	0.108	0.106	0.109	0.108	0.116	0.544	0.874	1.124
10	0.109	0.107	0.104	0.109	0.108	0.108	0.133	0.714	1.026	1.050
2	0.105	0.104	0.105	0.106	0.104	0.105	0.141	0.823	1.039	1.114
1	0.103	0.103	0.102	0.105	0.106	0.105	0.140	0.849	1.028	0.981

Ca37 (µg/ml)	Fluconazole (mg/L)									
	64	32	16	8	4	2	1	0.5	0.25	0.125
0	0.136	0.209	0.998	1.034	1.021	1.120	1.045	1.009	1.011	1.037
20	0.384	0.411	0.498	0.576	0.614	0.642	0.716	0.708	0.804	0.764
10	0.366	0.385	0.499	0.603	0.654	0.728	0.733	0.763	1.134	0.831
2	0.333	0.411	0.543	0.857	0.846	0.857	0.860	0.891	1.006	0.909
1	0.318	0.314	0.697	0.959	0.986	0.986	0.998	0.992	0.962	1.018

% of inhibition
100-40% 40-20% 20-0%

We analyze the additive effect of Ca37 mAb and the antifungal drugs amphotericin B and fluconazole following the EUCAST protocol. The combination of this mAb with antifungals reduced MIC values to half, even in the case of a fluconazole-resistant strain, UPV 93-153.

Figure 3. Additive effect of Ca37 mAb with antifungals. Growth of *C. albicans* CECT 13062 and UPV 93-153 strains in presence of (A) amphotericin B and (B) fluconazole, respectively, and the mAb. MIC values are marked in red. The grey scale indicates the percentage of inhibition.

4. Increased survival of *Galleria mellonella* infected with Ca37-treated yeasts

Candida albicans was treated with PBS, Ca37 or fluconazole for 18 h before been injected into the larvae, at a density of 5 x 10⁵ cells/larvae. Larvae receiving the yeast pre-treated with the mAb survived 2 days longer than the ones injected with PBS-treated fungus. The increase in survival was similar to that observed in the group receiving fluconazole treated yeasts.

Figure 4. Survival curves of *Galleria mellonella* larvae inoculated with *C. albicans* yeast pre-treated with the mAb, fluconazole or IgG1 isotype control (n=48). Data from a single representative experiment. *** p ≤ 0.0001 in comparison to non-treated *C. albicans*.

CONCLUSIONS

- Ca37 is able to inhibit *C. albicans* growth *in vitro*, to reduce the dose of antifungals and to increase survival of *Galleria mellonella* *in vivo*.
- The protein targeted by Ca37 mAb could be an attractive new target to fight against *C. albicans* infections.

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