Comparison of the effectiveness of caspofungin and liposomal amphotericin-B for the treatment of *C. tropicalis*-induced peritonitis in mice

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SUMMARY

In order to compare the effectiveness of liposomal amphotericin B (LAB) and caspofungin monotherapy in *Candida tropicalis*-induced peritonitis in an experimental mice model 56 healthy male BALB/c mice (10-12 weeks; 20-25 g) were divided into groups and *C. tropicalis* strains were intraperitoneally (IP) inoculated into mice groups except the control group. After the injection, three doses of LAB (0.5, 1.0, 2.0 mg/kg/day) and caspofungin (1.0, 2.0, 5.0 mg/kg/day) were administered to groups for five consecutive days, starting 48 h post-infection. The mice were then followed up for 14 days and killed by cervical dislocation. When their peritoneal fluid was examined, the difference in fungal growth between the treatment group and control group was significant ($p<0.05$). Evaluation of the treatment groups revealed that fungal growth decreased with increasing dose of the antifungal agent ($p>0.05$). There was no dose-related difference from mice which received LAB or those which received caspofungin in our experimental model. During our study, no death was detected despite the similar injection doses compared with other studies using *Candida* species. The results of this study suggest that *C. tropicalis* could have lower virulence, perhaps limited by natural immunity, and causes mortality at much higher doses.

Keywords: *Candida tropicalis*, fungal peritonitis, experimental mice model, liposomal amphotericin B, caspofungin.

■ INTRODUCTION

In recent years there has been an increase in the prevalence of invasive infections due to *Candida* species. The increase in number of immunsuppressed patients, surgical procedures, use of central venous catheters, and the use of broad-spectrum antibiotics have played a part in this increase.
Candida species are fungal pathogens that can cause mucosal and deep-tissue infections in humans. Exposure to *C. albicans*, *C. tropicalis*, *C. glabrata, C. parapsilosis* and *C. krusei* can lead to infections. A bloodstream infection is the most common type of invasive candidiasis. In addition, fungal peritonitis is a rare (but serious) intra-abdominal infection, and is difficult to diagnose by symptoms alone [1]. *C. albicans* is seen especially in surgical patients as well as those in intensive care units or patients undergoing hemodialysis. However, in recent years, there has been an increase in the prevalence of infection by species such as *C. tropicalis* [2]. In a retrospective study, a significant correlation was found between the shift from *C. albicans* to non-*C. albicans* strains and

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fluconazole consumption [3, 4]. In an important portion of these non-*C. albicans* species, resistance to azole-type antifungal agents was noted, so broad-spectrum antifungal drugs have important roles in these cases. Liposomal amphotericin B (LAB) and caspofungin show their fungicidal effects in *Candida* infections by a different mechanism of action. These drugs are used in azole-resistant or non-azole-resistant *C.albicans*/ non-*C. albicans* strains. Several studies have compared the efficacy of these antifungal drugs, especially in *C. albicans* infections.

We wished to:
1) focus on non-*C. albicans* infections and evaluate the virulence and mortality in a murine model of peritonitis;
2) compare the efficacy of caspofungin and LAB in the treatment of *C. tropicalis*-induced peritonitis.

■ MATERIALS AND METHODS

All procedures were conducted according to standards for the human handling and care of research animals set by Celal Bayar University Faculty of Medicine (Manisa, Turkey) and were approved by the Animal Research Ethics Committee of this institution.

Fifty-six healthy male BALB/c mice (10-12 weeks; 20-25 g) were used from our experimental animal laboratory and some were purchased from the Veterinary Research Laboratory. Mice were allowed free access to food and water. Animals were placed in plastic boxes, with seven animals per container.

Mice were randomized into eight groups according to the type and dose of antifungal treatment they were to receive (Table 1). Two control groups of seven mice were created. We administered the infective dose to one control group and the other control group was merely for observation under natural conditions. Antifungal-therapy groups of 21-rats each were classified by dose: liposomal amphotericin B (0.5, 1.0 and 2.0 mg/kg/day) and caspofungin (1.0, 2.0 and 5.0 mg/kg/day).

In this murine model, we used a *C. tropicalis* strain obtained from a child patient who was entering peritoneal dialysis in the intensive care unit of Celal Bayar University. The minimum inhibitory concentration (MIC) values for flucona-

zole (2 µg/mL), voriconazole (0.25 µg/mL) and itraconazole (0.064 µg/mL), respectively, were obtained according to CLSI Guidelines, 2015. This strain was stocked in a cryobank at -70°C. In preliminary studies and the actual study, this *C. tropicalis* strain was revived and fresh cultures prepared in Sabouraud dextrose broth and Sabouraud dextrose agar (Difco Laboratories, Detroit, MI, USA).

We undertook some preliminary tests before the actual study to ascertain the optimum dose to elicit infection. This dose was tested to cause peritonitis in 95% of mice without significant mortality. The deterioration in the general condition and death of mice, and subsequent presence of the etiologic agent in their organs, were considered important. The literature was searched and doses in similar studies determined. In preliminary studies, *Candida* solutions of 5×10^5, 10^6, 5×10^6, 10^7, 5×10^7, 10^8, 5×10^8, and 10^9 CFU/mL were tested. Observing the morbidity and mortality of mice, the optimal dose of *Candida* solution to induce peritonitis was determined to be 10^8 CFU/mL. Colonies were counted in a Thoma cell counting chamber (Thermo Scientific, Waltham, MA, USA). After ascertaining the infective dose, 10^8 CFU/mL *Candida* solution was injected intraperitoneally (i.p.) into mice except for one control group (Table 1). Then, solutions of liposomal amphotericin B and caspofungin in sterile distilled water were prepared, and both drugs administered (1 mL). Groups of infected mice were treated intraperitoneally with the appropriate antifungal agent for 5 consecutive days, starting 48 h after the induction of infection.

Mice were observed for 14 days, after which they

Table 1 - Mice groups (n = 7) designed according to antifungal agent and dose.

Group	Regimen
1	(a) Control group (not infected)
2	(b) Control group (infected but untreated)
3	Caspofungin (1 mg/kg/day)
4	Caspofungin (2.0 mg/kg/day)
5	Caspofungin (5.0 mg/kg/day)
6	Liposomal amphotericin B (0.5 mg/kg/day)
7	Liposomal amphotericin B (1.0 mg/kg/day)
8	Liposomal amphotericin B (2.0 mg/kg/day)

Table 2 - *C. tropicalis* growth in SDA in the groups (%).

Group	Number of mice with C. tropicalis growth*	%
Control group (infected but untreated)	7	100
Caspofungin (1 mg/kg/day)	1	14.3
Caspofungin (2.0 mg/kg/day)	0	0.0
Caspofungin (5.0 mg/kg/day)	0	0.0
Liposomal amphotericin B 0.5 mg/kg/day	3	42.9
Liposomal amphotericin B (1.0 mg/kg/day)	2	28.5
Liposomal amphotericin B (2.0 mg/kg/day)	0	0.0

*All groups contained 7 mice.

were killed by cervical dislocation. Samples of peritoneal fluid were removed aseptically and plated onto Sabouraud dextrose agar after incubation for 48 h in Sabouraud dextrose broth. Colonies were counted after 2 days of incubation in Sabouraud dextrose agar (SDA) at 37°C. Quantification of *Candida* density in peritoneal fluid was done to assess antifungal efficacy.

Data analyses were carried out using SPSS v13.0 (IBM, Armonk, NY, USA). The colonies on SDA were counted. The colony numbers are calculated as minimum (MIN) and maximum (MAX) because the number of mice is 7 in the groups. Differences between the median values of the two groups were assessed using the Mann-Whitney *U*-test; the difference in percentiles between the two groups was assessed by Fisher's exact test, and p <0.05 was considered significant.

■ RESULTS

After incubation of peritoneal fluid in Sabouraud dextrose agar, *C. tropicalis* growth in treatment and control groups was evaluated. All groups comprised seven mice, but not all of them showed fungal growth in the peritoneum (Table 2). The difference in fungal growth between the treatment group and control group was significant (p<0.05). Evaluation of the treatment groups revealed that fungal growth decreased with increasing dose of antifungal agent, but this difference was not significant (p>0.05).

Liposomal amphotericin B and caspofungin groups were compared with each other. The number of *Candida* colonies recovered in liposomal amphotericin B groups was less than that of caspofungin groups but this difference was not statistically significant (p>0.05). The number of

C. tropicalis colonies was higher in the untreated group, which was significant compared with the treatment group (p<0.05). No death occurred during the study. In LAB and caspofungin groups, there was no difference in effect related to dose. When the candida growth in SDA was evaluated, the density of the peritoneal candidiasis was significant compared with the control group.

■ DISCUSSION

The incidence of fungal infections has increased significantly since the 1980s, especially in immuncomproimized patients [5]. *Candida* species can colonize the oral cavity, gastrointestinal tract and vagina [6].

C. tropicalis can be seen especially in patients who have haematologic malignancies. In a study from India, *C. tropicalis* was the most prevalent pathogen in non-*C. albicans* species causing nosocomial invasive candidiasis (67-90%) [7]. However, fewer experimental studies have been conducted on non-*C. albicans* species than on *C. albicans.*

There are several choices for the treatment of candidal infections. Bailly investigated *Candida* infection in 2403 cases, and stated that an increase in MIC was because of fluconazole use [8]. Hence, other antifungal agents, such as liposomal amphotericin B and caspofungin, have become more popular. Liposomal amphotericin B has lower nephrotoxicity and good tolerability compared with other antifungal agents, and caspofungin has better tolerability than liposomal amphotericin B.

Several studies have compared liposomal amphotericin B and caspofungin in experimental

models. Barchiesi et al. showed that the fungicidal activity of these two agents against *Candida* infections is similar, and that they can be used as combination therapy [9, 10]. Studies comparing the efficacy of liposomal amphotericin B and caspofungin in peritonitis models of *C. albicans* have not shown any difference between these two drugs [11]. However, studies comparing the efficacy of these drugs against *C. tropicalis* infection are lacking.

Studies by Hossain et al. and Tunger et al. discussed combination therapy of LAB and caspofungin and found that, as the concentration of antifungal agent increased, a decrease in fungus concentration in tissue occurred [12, 13].

The fungal growth found in the present study could be related to the lower virulence of *C. tropicalis* compared with that in studies conducted using *C. albicans*. Koga-Ito and colleagues compared the virulence of *Candida* species [14]. Mice were infected (i.v.) with *Candida* isolates and they evaluated tissues 6 h as well as 3, 7, 14 and 21 days after inoculation. After 7 days, the *C. tropicalis* concentration in tissues decreased except for that in the brain. After 14 days, especially in the spleen, the *C. tropicalis* concentration could not be detected. Also, the group infected with *C. tropicalis* had longer survey than the *C. albicans* group which supports the mortality results of our study.

An extensive study was done by Arendrup et al. using 207 mice and eight *Candida* species [5]. The virulence of and death caused by *C. albicans* were higher than those caused by *C. tropicalis*.

The most important finding in our experimental model was the significant difference between the treatment group and control group which had been infected but not treated. In accordance with the literature, there was no dose-related difference from mice who received LAB or those who received caspofungin in our experimental model. During our study, no death was detected despite the similar injection doses compared with other experimental studies using *Candida* species.

This study's result made us think that *C. tropicalis* could have lower virulence, maybe limited with the natural immunity and cause mortality in much higher doses. So there is a need for larger series of experimental and clinical studies about the invasive infections of non-albican *Candida* species.

■ REFERENCES

[1] Kao A.S., Brandt M.E., Pmitt W.R., et al. The epidemiology of candidemia in two United States cities: results of a population based active surveillance. *J. Clin. Infect. Dis.* 29, 1164-1170, 1999.
[2] Zarrin M. Invasive candidiasis; a review article. *Jundish J. Microbiol.* 2, 1, 1-6, 2009.
[3] Bassetti M., Righi E., Costa A., et al. Epidemiological trends in nosocomial candidemia in intensive care. *BMC Infect. Dis.* 10, 6, 21, 2006.
[4] Oberoi J.K. Invasive candidiasis. JIMSA 1, 23, 2010.
[5] Arendrup M., Horn T., Frimodt-Møller N. *In Vivo* pathogenicity of eight medically relevant *Candida* Species in an animal model. *Infection.* 30, 286-291, 2002.
[6] Eggimann P., Garbino J., Pittet D. Epidemiology of *Candida* species infections in critically ill non- immunosuppressed patients. *Lancet Infect. Dis.* 3, 685-702, 2003.
[7] Kothari A., Sagar V. Epidemiology of *Candida* bloodstream infections in a tertiary care institute in India. *Indian J. Med. Mic.* 27, 171-172, 2009.
[8] Bailly S. Impact of antifungal prescription on relative distribution and susceptibility of *Candida* spp. - Trends over 10 years. *J. Infect.* 72, 1, 103-111, 2016.
[9] Barchiesi F., Spreghini E., Tomassetti S., et al. Comparison of the fungicidal activities of caspofungin and amphotericin B against *Candida glabrata*. *Antimicrob. Agents Chemother.* 49, 12, 4989-4992, 2005.
[10] Barchiesi F., Spreghini E., Tomassetti S., et al. Effects of caspofungin against *Candida guilliermondii* and *Candida parapsilosis*. *Antimicrob. Agents Chemother.* 50, 8, 2719-2727, 2006.
[11] Pelz R.K., Hendrix C.W., Swoboda S.M. Double-blind placebo-controlled trial of fluconazole to prevent candida infections in critically ill surgical patients. *Ann. Surg.* 233, 542, 2001.
[12] Hossain M.A., Reyes G.H., Long L.A., et al. Efficacy of caspofungin combined with amphotericin B against azole-resistant *Candida albicans*. *J. Antimicrob. Chemother.* 51, 1427-1429, 2003.
[13] Tunger O., Bayram H., Degerli K., et al. Comparison of the efficacy of combination and monotherapy with caspofungin and liposomal amphotericin B against invasive candidiasis. *Saudi Med. J.* 29, 5, 728-733, 2008.
[14] Koga-Ito C.Y., Komiyama E.Y., Martins C.A., et al. Experimental systemic virulence of oral *Candida dublinensis* isolates in comparison with *Candida albicans, Candida tropicalis* and *Candida krusei*. *Mycoses.* 54, 278-285, 2010.