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 immunosuppressed 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
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 fluconazole (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 $5 \times 10^5$, $10^6$, $5 \times 10^6$, $10^7$, $5 \times 10^7$, $10^8$, $5 \times 10^8$, 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>
<thead>
<tr>
<th>Group</th>
<th>Regimen</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(a) Control group (not infected)</td>
</tr>
<tr>
<td>2</td>
<td>(b) Control group (infected but untreated)</td>
</tr>
<tr>
<td>3</td>
<td>Caspofungin (1 mg/kg/day)</td>
</tr>
<tr>
<td>4</td>
<td>Caspofungin (2.0 mg/kg/day)</td>
</tr>
<tr>
<td>5</td>
<td>Caspofungin (5.0 mg/kg/day)</td>
</tr>
<tr>
<td>6</td>
<td>Liposomal amphotericin B (0.5 mg/kg/day)</td>
</tr>
<tr>
<td>7</td>
<td>Liposomal amphotericin B (1.0 mg/kg/day)</td>
</tr>
<tr>
<td>8</td>
<td>Liposomal amphotericin B (2.0 mg/kg/day)</td>
</tr>
</tbody>
</table>
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 immunocompromised 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. Baille 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

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

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

*All groups contained 7 mice.*
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 Tünger 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.

ACKNOWLEDGEMENT
All authors declare that there is no conflict of interest.

Conflicts of interest: none

REFERENCES