Immunomodulatory Effects of Voriconazole and Caspofungin on Human Peripheral Blood Mononuclear Cells Stimulated by *Candida albicans* and *Candida krusei*

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Abstract: *Background: Candida* infections are frequently associated with high morbidity and mortality rates in immunosuppressed patients. T cell–mediated and phagocytic immunity are the primary protective immune responses against fungal infections. Antifungal agents such as voriconazole and caspofungin enter phagocytic cells and lead to various intracellular activities. In this study, the authors aimed to investigate the immunomodulatory effects of voriconazole and caspofungin on human peripheral blood mononuclear cells (PBMC) stimulated by *Candida albicans* and *Candida krusei*. *Methods:* Human PBMC isolation was performed by Ficoll-hypaque density-gradient centrifugation method. Cell proliferation was assessed by colorimetric method using MTT. The cytokine levels in the human PBMC culture supernatants stimulated by *C. albicans* and *C. krusei* were determined by enzyme-linked immunosorbent assay. *Results:* The addition of voriconazole and caspofungin lead to proliferation of PBMC. In the presence of voriconazole and caspofungin, the levels of IL-2, IFN-γ and IL-6 remarkably increased in PBMC stimulated by *C. albicans* and *C. krusei*. However, the combination of antifungal drugs and PBMC stimulated by *Candida* species did not increase the levels of TGF-β and IL-10. *Conclusions:* The results indicate that voriconazole and caspofungin have immunomodulatory effects on human PBMC stimulated by *Candida* species. The interaction between antifungal drugs and PBMC stimulates Th1-type cytokine secretion. Cytokine stimulation from immune cells can assist in the elimination of fungal pathogens. Therefore, during the treatment of fungal infection, putative immunomodulatory effects of antifungal agents should be taken into account.

Key Indexing Terms: Immunomodulatory effects; Voriconazole; Caspofungin; *Candida*. **[Am J Med Sci 2014;348(3):219–223.]**

Candida infections are associated with high morbidity and mortality rates in immunosuppressed patients.[1] Although *Candida albicans* is the most frequently isolated species in patients with candidemia, infection rates caused by other *Candida* species such as *Candida krusei* is increasing. The development of resistance against most of the earlier antifungal agents among *Candida* species has made the treatment of these infections more difficult.[2] The development of echinocandins such as caspofungin and newer triazole agents such as voriconazole has become important in the treatment of infections caused by resistant *Candida* species.

Voriconazole (VRC) is an antifungal triazole with activity against medically important fungal infections in immunocompromised patients.[3] VRC inhibits the synthesis of ergosterol by inhibiting the lanosterol 14α-demethylase enzyme.[4] Caspofungin, the 1st clinically used echinocandin, is a member of a new class of antifungal antibiotics.[5] Caspofungin inhibits fungal β-1,3 glucan synthesis and is effective in the treatment of mucosal and invasive fungal infections.[6]

T cell–mediated immunity and phagocytic immunity are the primary protective immune responses against fungal infections. Phagocytic cells such as monocytes and monocyte-derived macrophages are known to be important in clearing fungal infections. Phagocytes are activated by pattern recognition receptors that recognize fungus and trigger expression of cytokines that modulate the antifungal host defense.[7] Peripheral blood mononuclear cells (PBMCs) are important cells in host defense against fungal infections, especially in invasive infections. Interactions with these cells may influence the efficacy of antifungal agents.[8] The modulatory effects of antifungal agents against on the host immune response are not well understood. Recently, the immunomodulatory effects of antifungal agents, especially newer antifungals such as azoles and echinocandins, have been described.[9] Antifungal agents either alone or combined with phagocytes are effective in inhibiting fungal growth.[7] Antifungal agents such as VRC and caspofungin enter phagocytic cells and cause intracellular activities.[10] Therefore, it is suggested that antifungal agents have also direct immunomodulatory effects. A better understanding of the immunomodulatory effects of antifungal agents may provide new strategies to improve the host immune response in invasive fungal infections. The aim of this study was to evaluate the immunomodulatory effects of VRC and caspofungin on human PBMC stimulated by *C. albicans* and *C. krusei*.

METHODS

Preparation of Human PBMC

Heparinized blood was collected from 3 healthy male blood donors. The age range of the donors was between 20 and 40. All samples were negative for HBsAg, HCV antibody and HIV antibody enzyme-linked immunosorbent assay (ELISA) tests. PBMC isolation was performed by Ficoll-hypaque density-gradient centrifugation method. After centrifugation, buffy coats were collected and washed in phosphate-buffered saline (PBS; Gibco, Darmstadt, Germany) for 3 times and resuspended at a concentration of 2×10^6 cells per milliliter with complete RPMI 1640 medium containing 2 mM l-glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, 0.05 mM 2-mercaptoethanol and supplemented with 10% fetal calf serum (Gibco, Germany). Cell viability was evaluated as 95% by the trypan blue exclusion

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test. Antifungal susceptibility of *Candida* isolates were performed using the broth microdilution test as recommended by the CLSI, document M27-A2.

Yeast Strains

C. albicans ATCC 10231 and *C. krusei* ATCC 6258 were used in this study. *Candida* suspensions were adjusted at the concentration of 10^6 CFU/mL. Afterward, *Candida* cells were inactivated by heating at 60°C for 30 minutes.

Drug Preparation

The lyophilized powders of Vfend (Pfizer, Sandwich, UK) and Cancidas (Merck, Darmstadt, Germany) were reconstituted in dimethyl sulfoxide, diluted with sterile water.

The MIC Values of Antifungal Agents

The MICs of VRC and caspofungin were measured by the broth microdilution test as recommended by the CLSI, document M27-A2.[11] MICs of VRC/caspofungin for *C. albicans* were 1 μg/mL and 2 μg/mL, respectively. For *C. krusei*, MICs of VRC/caspofungin were 0.25 μg/mL and 0.5 μg/mL, respectively.

Incubation of PBMC With *Candida* Species and Antifungal Agents

PBMCs (2×10^6) were delivered in culture flasks. PBMCs were incubated with heat-inactivated *C. albicans* or *C. krusei* (10^6 CFU/mL) at an effector:target ratio of 1:1 in the presence or absence of VRC or caspofungin. Concentrations of 1× MIC of VRC and caspofungin were added to the wells. Control culture flasks contained only PBMC or only antifungals. All flasks were studied for 3 times. Culture flasks were incubated in 5% CO_2 for 24 and 48 hours at 37°C. At the end of each selected time period, the culture supernatants were removed and stored at −30°C until used.

Cell Proliferation

The proliferation of PBMCs was assessed by colorimetric method using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (Sigma, Steinheim, Germany). PBMCs were washed and incubated with MTT for 24, and after 48 hours, they were treated with *C. albicans* or *C. krusei* in the presence of VRC or caspofungin. Then, 0.5 mg/mL MTT-media solution were added to each well for 4 hours at 37°C. After centrifugation, DMSO was added. The absorbance was read at 560 nm with a spectrophotometer.

Cytokine Secretion

Levels of cytokines such as IFN-γ, IL-2, IL-6, IL-10 and TGF-β were determined by specific ELISA techniques according to the manufacturer's instructions (Biosource, California). The concentration of cytokines was determined spectrophotometrically. The absorbance was read at 450 nm. A standard curve was created using cytokine standards. The cytokine concentrations for unknown samples were calculated according to the standard curve.

Statistical Methods

The results were analyzed using the 1-way analysis of variance. The Bonferroni test was used as *post hoc* analysis. $P < 0.05$ was considered as statically significant.

RESULTS

The addition of VRC and caspofungin leads to the proliferation of PBMC stimulated by *Candida* (Figure 1). Both of the antifungal agents lead to remarkable proliferation in PBMCs stimulated by *C. albicans* and *C. krusei*. The PBMC proliferation produced by caspofungin was determined at much higher levels after 48 hours.

In the presence of VRC and caspofungin, the levels of IL-2 increased remarkably in PBMC stimulated by *C. albicans* and *C. krusei* (Figure 2) ($P < 0.05$). Moreover, the addition of VRC and caspofungin to PBMCs stimulated by *C. albicans* resulted in more significant increase in the IL-2 levels compared with the PBMCs stimulated by *C. krusei* ($P < 0.001$).

VRC and caspofungin caused a statistically significant increase in the release of IFN-γ from PBMCs stimulated by *C. albicans* and *C. krusei* at the 24th and 48th hours compared with the wells without any antifungal agent (Figure 3) ($P < 0.05$). Caspofungin produced the most remarkable increase in the IFN-γ levels at the 48th hour in the presence of *C. albicans* ($P < 0.001$).

The addition of VRC and caspofungin to PBMCs stimulated by *C. albicans* and *C. krusei* caused a statistically significant increase in the IL-6 levels compared with the PBMC wells without any antifungal agents at the 24th and 48th hours (Figure 4) ($P < 0.05$). *C. krusei* caused much more increase in the IL-6 levels in PBMCs in the presence of antifungal agents at the 24th and 48th hours compared with the wells with *C. albicans*. However, the increase in the wells with VRC after 24 hours and in the wells with VRC and caspofungin after 48 hours was found to be statistically significant in the presence of *C. krusei* compared with the wells with *C. albicans* ($P = 0.001$, $P = 0.001$, $P = 0.027$, respectively).

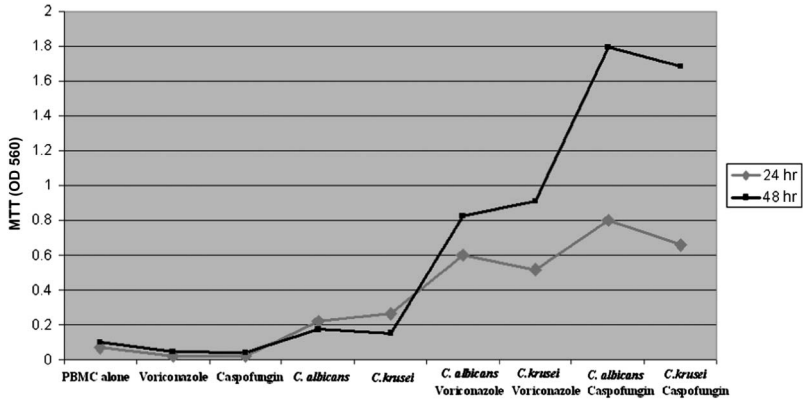

FIGURE 1. MTT assay for PBMCs proliferation analysis.

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