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## *Candida albicans* Infections in Renal Transplant Recipients: Effect of Caspofungin on Polymorphonuclear Cells<sup>∇</sup>

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**This study aimed to compare the caspofungin immunomodulating activities against *Candida albicans* on polymorphonuclear cells (PMNs) from renal transplant recipients (RTRs) and healthy subjects (HSs). RTR PMNs showed a significantly reduced fungicidal activity compared with that of HS PMNs. Addition of caspofungin to RTR PMNs significantly potentiated the yeast intracellular killing rate, achieving values similar to those observed for HS PMNs. These data show that caspofungin is suitable for invasive candidiasis treatment in patients with immune system-impaired components.**

Renal transplant recipients (RTRs) are highly susceptible to invasive fungal infections (IFIs) mainly due to *Candida* spp., which continue to be the most frequent cause of death (60%) in the posttransplant period (1, 2, 13–15, 21). In fact, *Candida* spp. may easily find ideal conditions for growing and invading tissues in relation with both long-term immunosuppressive treatment and decreased defense mechanisms (1, 7).

Among newer antifungals, echinocandins such as caspofungin can be used for IFI treatment, thanks to their favorable pharmacodynamic/pharmacokinetic characteristics, lower toxicity, and immunopharmacologic mode of action (4, 6, 9–12, 20, 23). This study aimed to compare the immunomodulating activities of caspofungin against *Candida albicans* on PMNs from RTRs and healthy subjects (HSs).

(This study was presented in part at the XLVII ERA-EDTA Congress, abstr. Sa283, Munich, Germany, 25 to 28 June 2010).

All patients participating in this study gave their written informed consent. Blood samples were obtained from 30 HSs (controls) and from 54 RTRs, 31 males and 23 females (mean age, 53.9 years), without any evidence of infection, being followed at Ivrea Hospital (Turin, Italy). The mean time since transplantation was 83.94 months (range, 7 to 248 months; for 2/54 patients, less than 12 months since transplant), and the primary renal diseases which infected these patients were as follows: nephroangiosclerosis ( $n = 16$ ), chronic glomerulonephritis ( $n = 6$ ), interstitial nephritis ( $n = 5$ ), polycystic kidney disease ( $n = 4$ ), diabetic nephropathy ( $n = 4$ ), chronic renal failure ( $n = 10$ ), and other ( $n = 9$ ). The mean serum creatinine level at the time of the study was  $1.7 \pm 0.8$  mg/dl. Posttransplant immunosuppressive treatment used included the following: for the majority of patients, tacrolimus (FK), mycophenolate mofetil (MMF), and prednisone (P); for 2 patients, cyclosporine (CyA) and MMF; for 7 patients, CyA, P, and

sirolimus; for 3 patients, P and FK; for 4 patients, FK and MMF; for 5 patients, FK alone; and for 1 patient, CyA alone.

A clinical *C. albicans* strain, isolated from blood and identified by biochemical methods, was subcultured on Sabouraud dextrose agar (Oxoid S.p.A., Milan, Italy) to ensure viability and purity (20). Caspofungin acetate (Merck Sharp & Dohme Ltd., Hoddesdon, United Kingdom) was dissolved in pyrogen-free water and stored at  $-20^{\circ}\text{C}$ .

Antifungal susceptibility testing was performed with an inoculum of  $10^3$  CFU/ml, in accordance with CLSI M27-A3 (5), and an inoculum of  $10^6$  CFU/ml, used to perform tests with phagocytes.

PMNs separated from lithium-heparinized venous blood using Ficoll-Paque (Pharmacia S.p.A., Milan) were suspended in RPMI 1640 medium ( $10^6$  PMNs/ml) (Gibco Laboratories, NY) as described previously (3). PMN viability was greater than 95%.

Both phagocytosis of radiolabeled *C. albicans* ( $[^3\text{H}]\text{uracil}$  [specific activity, 1,270 GBq/mmol; NEN Life Science Products, Milan]) and intracellular blastoconidia killing by PMNs were investigated by incubation of yeast cells ( $10^6$  yeast cells/ml) and PMNs (1:1 ratio) at  $37^{\circ}\text{C}$  in a shaking water bath for 30, 60, and 90 min in the presence of one times the MIC ( $2 \mu\text{g/ml}$ ), one-half times the MIC, and one-fourth times the MIC of caspofungin. Drug-free controls were included. Phagocytosis and intracellular killing were assessed by the methods described previously (3, 20). Radioactivity was expressed as counts per minute (cpm) per sample. The percentage of phagocytosis at a given sampling time was calculated as follows: percentage of phagocytosis =  $[(\text{number of cpm in PMN pellet})/(\text{number of cpm in total fungal pellet})] \times 100$  (3, 20). The PMN killing values were expressed as the survival index (SI), which was calculated by adding the number of surviving yeast cells at time zero to the number of survivors at time  $x$  and dividing by the number of survivors at time zero (3, 20). According to this formula, if fungal killing was 100% effective, the SI would be 1.

Results were expressed as the means  $\pm$  the standard errors

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TABLE 1. Effect of 1× MIC of caspofungin against *C. albicans* on intracellular killing of PMNs from HSs and RTRs

Time (min)	Mean % phagocytosis ± SEM		SI ± SEM (%) <sup>b</sup>	
	Controls	PMNs treated with caspofungin (2 µg/ml)	Controls	PMNs treated with caspofungin (2 µg/ml)
<b>HSs</b>				
30	77.1 ± 0.67	77.2 ± 0.76	1.54 ± 0.03 (46)	1.29 ± 0.07 (71) <sup>a</sup>
60	75.8 ± 0.11	75.1 ± 0.11	1.53 ± 0.02 (47)	1.28 ± 0.11 (72) <sup>a</sup>
90	75.1 ± 0.32	75.9 ± 0.09	1.52 ± 0.03 (48)	1.25 ± 0.07 (75) <sup>a</sup>
<b>RTRs</b>				
30	74.9 ± 0.74	74.3 ± 0.02	1.54 ± 0.1 (46)	1.44 ± 0.09 (56)
60	76.4 ± 0.46	76.1 ± 0.15	1.65 ± 0.14 (35)	1.42 ± 0.11 (58) <sup>a</sup>
90	76.4 ± 0.58	76.7 ± 0.31	1.74 ± 0.08 (26)	1.33 ± 0.11 (67) <sup>a</sup>

<sup>a</sup> Significantly different from controls ( $P < 0.01$ ).

<sup>b</sup> Values shown in parentheses represent the percentages of yeast cells killed by PMNs in the absence and in the presence of the antifungal drug.

of the means (SEM) from 10 separate experiments, each performed in quadruplicate. Statistical evaluation of the differences between test and control results was performed by Tukey's test. A  $P$  value of  $<0.05$  was considered significant.

RTR PMNs showed phagocytosis toward *C. albicans* similar to that registered for HS PMNs (Table 1), whereas they displayed significant reduced fungicidal activity within 90 min ( $P < 0.01$ ), confirming our previous findings, which show that in RTRs, the number of PMNs was normal, but several metabolic and functional alterations were observed, resulting in a high incidence of infections in these patients (19).

As observed for other systemic antifungal drugs (8, 16, 19, 20), no synergistic effect between PMNs and caspofungin on phagocytosis toward *C. albicans* in RTR or HS PMNs (Table 1) throughout the observation period occurred, indicating that caspofungin did not adversely interfere with PMN phagocytic capacity, probably due to a dramatic effect on the integrity of the yeast cell wall. The expression of surface molecules that link to cleavage products of complement could be altered with direct consequences for phagocytosis (8, 16, 20).

On the contrary, the addition of caspofungin significantly ( $P < 0.01$ ) elicited fungicidal activity against intracellular *C. albicans* by HS PMNs compared to that observed in drug-free controls (Table 1). Analogously, when caspofungin was added to RTR PMNs, their depressed fungicidal activity was completely restored, with rates quite similar to those of HS PMNs, indicating that caspofungin synergized for fungal killing with PMNs being able to kill *C. albicans* at values significantly higher than those observed in drug-free RTR PMNs ( $P < 0.01$ ) (Table 1). A similar pattern was also detected when RTR PMNs and yeast cells were exposed to sub-MICs of caspofungin (data not shown), indicating the absence of a drug dose-dependent effect. The increased PMN killing activity observed at various caspofungin concentrations is related to a direct drug action on both *C. albicans* and phagocytes, as previously demonstrated (20) by the following: caspofungin is able both to unmask a fungal virulence factor in the yeast cell wall, making intracellular blastoconidia more susceptible to PMN lytic mechanisms, and to enter phagocytes remaining available in a biologically active form against proliferating yeast cells.

Taken together, the results of this study provide evidence that caspofungin is able to elicit the depressed phagocytic response of PMNs from RTRs, with the potential to totally

restore their primary functions *in vitro*. These findings support and could explain the improvement of survival in neutropenic high-risk patients with IFIs undergoing an empirical treatment with caspofungin versus liposomal amphotericin B, as described in the literature (17, 18, 24). In spite of the high cost of caspofungin, which nowadays limits its use to azole-resistant cases of candidal infections (22, 23), all these data permit the conclusion that caspofungin, in addition to its antifungal activity, possesses immunomodulating properties that make it highly suitable for fungal infection treatment in patients with impaired components of the immune system, which represent a high-risk population.

To better assess these encouraging findings, further studies by testing both other *Candida* clinical isolates and other immunocompromised patients are needed.

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We have no conflicts of interest to declare.

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