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Role of caspofungin in restoring the impaired phagocyte-dependent innate immunity towards *Candida albicans* in chronic haemodialysis patients☆

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ABSTRACT

Phagocyte-dependent cellular immunity in chronic kidney disease patients undergoing haemodialysis treatment is frequently impaired owing to the uraemic state, resulting in an intrinsic susceptibility to developing invasive fungal infections with high mortality rates. Since synergism between phagocytic cells and antifungal drugs may be crucial for successful therapy, the aim of this study was to evaluate the effects exerted by caspofungin (CAS) on the functional activities of polymorphonuclear cells (PMNs) in haemodialysed patients (HDs) towards *Candida albicans* compared with those of PMNs from healthy subjects (HSs). PMNs were separated from venous blood samples of 66 HDs and 30 HSs (as controls), and measurement of phagocytic and intracellular fungicidal activities of HD-PMNs and HS-PMNs was performed in the presence of CAS at the minimum inhibitory concentration (MIC) and at sub-MICs. CAS-free controls were also included. In the drug-free test condition, no significant difference between the phagocytic activity of HD-PMNs and HS-PMNs was detected. In contrast, a progressive decline in the intracellular killing activity of HD-PMNs against proliferating yeasts was observed. CAS at MIC and sub-MIC levels was able to improve significantly the intracellular fungicidal activity of HD-PMNs against *C. albicans*, restoring their functionality. These findings provide evidence that CAS exerts a synergistic effect on HD-PMNs against *C. albicans*, being able to strength the depressed intracellular killing activity. These results corroborate the use of CAS as an effective therapeutic option for the treatment of invasive fungal infections in HDs, in whom even a marginal influence of antifungal drugs on host response may have a relevant effect.

1. Introduction

Candida albicans infections represent a serious clinical complication in patients with chronic kidney disease (CKD) undergoing haemodialysis treatment and are associated with a high mortality rate [1,2]. The greater risk of infection in this population is caused by impaired host immunity through uraemia, which interferes with T-cell and B-cell function, macrophage phagocytosis and antigen presentation [3–6]. Systemic antifungals have been extensively used for prophylaxis and/or as empirical therapy in high-risk populations; amphotericin B deoxycholate has so far been considered the target treatment for proven invasive fungal infections. To date, however, this treatment has been replaced with new triazoles, i.e. fluconazole and voriconazole, and echinocandins such as caspofungin (CAS), micafungin and anidulafungin. In fact, echinocandins, thanks to their high clinical efficacy, broad-spectrum activity against *Candida* spp. and several moulds, and low rate of treatment-related adverse events, represent an effective treatment option in patients with refractory invasive *Candida* and *Aspergillus* infections [7–10] and as empirical antifungal therapy in neutropenic patients [11]. In addition, recent evidence suggests that antifungal drugs may stimulate or alter the host immune response by mechanisms that may result in enhanced fungal clearance [7–10,12–14]. Hence, antifungal drugs with immune-enhancing properties that positively influence phagocyte activities may be crucial for resolution of fungal infections amongst critically ill immunocompromised patients. Recently, we have reported that CAS has interesting immunomodulating effects on phagocyte-mediated host responses to *C. albicans* by improving the intracellular fungicidal activity

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of human polymorphonuclear cells (PMNs) from healthy subjects [15]. The main purpose of this study was to evaluate the potential PMN–CAS interaction in eradicating *C. albicans*, the most common life-threatening fungal pathogen in immunocompromised hosts, by studying both the phagocytic and fungicidal activities of PMNs in CKD patients undergoing chronic haemodialysis treatment compared with those of PMNs from healthy subjects.

2. Materials and methods

2.1. Patients

All patients participating in this study gave written informed consent. Blood samples were collected from 30 healthy subjects (HSs) as controls. Blood samples were also collected from 66 haemodialysed patients (HDs), without any evidence of infection, followed at the Dialysis Center of the Ivrea Hospital (Turin, Italy), comprising 42 males and 24 females (mean age 69.3 years, range 31–89 years). The mean time on dialysis was 52.8 months (range 1–359 months) and the causes of renal failure were as follows: chronic glomerulonephritis (8 cases); nephroangiosclerosis (18 cases); polycystic kidney disease (2 cases); diabetic nephropathy (12 cases); chronic renal failure (18 cases); kidney myeloma (2 cases); interstitial nephritis (1 cases); and other (5 cases). The mean normalised dose of dialysis/treatment (Kt/V single-pool = 1.44) and the protein catabolic rate (PCR = 1.15) indicated an adequate dialysis prescription and nutrition. The dialyser membrane was modified cellulose without reuse.

2.2. Yeasts

A clinical *C. albicans* strain isolated from blood and identified by biochemical methods was subcultured on Sabouraud dextrose (SAB) agar (Oxoid S.p.A., Milan, Italy) to ensure viability and purity. Yeast cultures consisted entirely of blastoconidia and had a slight tendency to differentiate into pseudohyphae during the course of the experiments.

2.3. Antifungal activity of caspofungin against Candida albicans

Caspofungin acetate was kindly supplied by Merck Sharp & Dohme Ltd. (Hoddesdon, UK). Stock solutions of the drug were prepared in pyrogen-free distilled water and were stored at −20 °C. Antifungal susceptibility testing was performed in RPMI 1640 medium (0.2% glucose) (Sigma, Milan, Italy), buffered to pH 7.0 with 0.165 M morpholinepropanesulfonic acid (MOPS) (Sigma), with an inoculum of 10^3 colony-forming units (CFU)/mL in accordance with Clinical and Laboratory Standards (CLSI) guidelines [16] for minimum inhibitory concentration (MIC) determination and an inoculum of 10^6 CFU/mL to perform tests with PMNs.

2.4. Polymorphonuclear cells

PMNs separated from lithium-heparinised venous blood using Ficoll–Paque™ (Pharmacia S.p.A., Milan, Italy) were suspended in RPMI 1640 medium as previously described in detail [15,17–21]. PMN viability, determined by trypan blue exclusion, was >95%.

2.5. Radioactive labelling protocol

A total of 200 μL of frozen culture was placed into fresh SAB broth containing 150 μL of ^{3}H-uracil (specific activity 1270 GBq/mmol) (NEN Life Science Products, Milan, Italy) at 37 °C for 4 h. Radiolabelled *Candida* were centrifuged several times with SAB broth, re-suspended in fresh medium and adjusted to yield 10^6 CFU/mL as confirmed by colony counts in triplicate.

2.6. Effect of caspofungin on phagocytosis and intracellular killing

The effect of CAS on the phagocytosis of radiolabelled *C. albicans* by PMNs was investigated by incubating yeasts (10^6 CFU/mL) and PMNs (10^6 cells/mL) at 37 °C in a shaking water-bath for periods of 30, 60 or 90 min in the presence of 1× MIC, 0.5× MIC and 0.25× MIC of CAS. CAS-free controls were also included. After 30, 60 or 90 min, phagocytosis was assessed. PMNs were centrifuged twice at 200 × *g* for 5 min to remove free blastoconidia and were suspended in sterile water for 5 min. Then, 100 μL samples were placed in scintillation fluid (Atomlight; NEN Life Science Products) and were counted by spectrophotometry. Radioactivity was expressed as counts per minute (cpm) per sample. The percentage of phagocytosis at a given sampling time was calculated as follows: % phagocytosis = [(cpm in PMN pellet)/(cpm in total fungal pellet)] × 100 [15]. Intracellular killing was investigated by incubating yeast cells and PMNs (1:1 ratio) for 30 min to allow phagocytosis to proceed. The PMN–yeast cell mixtures were centrifuged at 200 × *g* for 5 min and were washed to remove extracellular blastoconidia. An aliquot of PMNs was lysed by adding sterile water and then intracellular viable yeast cell counting was performed (time zero). PMNs were incubated further with 1× MIC, 0.5× MIC and 0.25× MIC of CAS and at time *x* (30, 60 and 90 min) viable counts were measured in the same way. Killing values were expressed as a 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. According to this formula, if fungal killing was 100% effective, the SI would be 1 [15].

2.7. Statistical analysis

Statistical analysis was performed using GraphPad Prism v.3.00 for Windows (GraphPad Software, San Diego, CA). Results are expressed as mean ± standard error of the mean for 10 separate experiments each performed in quadruplicate. Evaluation of differences between test and control results was performed using Student's unpaired *t*-test. *P*-values of <0.05 were considered statistically significant.

3. Results

MICs of CAS for the *C. albicans* clinical strain were 0.5 μg/mL with an inoculum of 10^3 CFU/mL and 2 μg/mL with an inoculum of 10^6 CFU/mL. Both phagocytosis and intracellular killing of PMNs from HSs and HDs against *C. albicans* were evaluated in the presence of 2 μg/mL of the drug (MIC). In all experiments, the viability of PMNs remained unchanged throughout. In the drug-free test condition, there was no significant difference between the phagocytic activities of HD-PMNs and HS-PMNs during the 90-min incubation time (Table 1). In contrast, HD-PMNs were less effective in eradicating proliferating yeasts than HS-PMNs, with reduced intracellular fungicidal activity. In fact, blastoconidia were only partially killed by HD-PMNs, with SI values of 1.70, 1.75 and 1.73 at 30, 60 and 90 min of incubation, respectively, whereas a significantly higher fungicidal effect was detected for HS-PMNs at all incubation times, with SI values of 1.54, 1.53 and 1.52, respectively (Table 1).

CAS at 1× MIC did not influence significantly the phagocytic activity of PMNs in HD or HS groups, as phagocytosis rates similar to drug-free HD controls and to those observed in HS-PMNs were detected (Table 2). Conversely, CAS at 1× MIC added to PMNs after phagocytosis had occurred significantly improved the intracellular fungicidal activity of PMNs in both groups, especially after