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Fungicidal activity of anidulafungin in serum from patients does not correlate to its susceptible breakpoint against Candida spp.

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Sir,

Echinocandins, such as anidulafungin, are now considered a primary treatment for patients with suspected candidiasis or candidaemia.1 Common Candida spp. are highly susceptible to these antifungal agents and >99% of isolates are inhibited by ≤2 mg/L, the current susceptible breakpoint.2 In vitro time-kill studies find that echinocandins are fungicidal against Candida spp. at concentrations achieved in serum.3 A major concern with the results from in vitro studies is the absence of testing in the presence of serum proteins. The echinocandins are highly protein bound (>95%), which significantly reduces their antifungal activity.4 The current CLSI susceptible breakpoint for the echinocandins was based upon MIC distribution data, pharmacodynamic (PD) models and results from clinical efficacy studies.5 Since there was a paucity of data concerning the impact of protein binding on human PDs, we initiated an ex vivo study to analyse the effect of serum, from patients receiving anidulafungin, on Candida spp. utilizing time–kill methodology.

Adult, non-neutropenic patients beginning empirical treatment with anidulafungin for presumed candidaemia/candidiasis were enrolled into this study following written informed consent. The study was approved by the hospital investigational review board and all guidelines for human research were followed in the conduct of this trial. All patients were treated with an initial loading dose of 200 mg of anidulafungin followed by 100 mg maintenance doses once daily. A venous blood sample was obtained prior to initiation of anidulafungin therapy. A set of samples was also collected at the end of a 2 h infusion (peak) and prior to the next infusion (trough) following multiple

References
17. Souli M, Kontopidou M, Papadomichelakis E. Clinical experience of serious infections caused by Enterobacteriaceae producing VIM-1
doses of anidulafungin. The concentration of anidulafungin in these sera was measured by a validated reverse-phase HPLC assay.6

The MIC of anidulafungin was determined for clinical isolates of Candida albicans (MICs of 0.01 and 0.03 mg/L), Candida glabrata (MICs of 0.06, 0.25, 0.5, 1.0, 2.0 and 4.0 mg/L), Candida parapsilosis (MICs of 0.5, 1.0, 2.0 and 4.0 mg/L) and Candida krusei (MIC of 0.06 mg/L) by broth microdilution following CLSI methodology.7 The MIC was defined as the lowest drug concentration to produce a prominent decrease (score of 2) in turbidity (MIC). Isolates of C. parapsilosis (ATCC 22019) and C. krusei (ATCC 6258) were utilized as control strains.

Fungicidal activity in serum was determined for these Candida spp. by a modification of the CLSI standard for time–kill curve methodology for bacteria. In brief, peak serum samples (225 µL) from patients were plated in microtitre plates, inoculated with 10⁶ cfu/mL and incubated at 35°C. Aliquots were removed at 0, 2, 6 and 24 h and plated onto Sabouraud dextrose agar plates. Plates were incubated for 24 or 48 h and colonies counted. The median number of cfu/mL at each time period was determined and used to produce time–kill curves. Fungicidal activity was defined as a ≥3 log reduction in fungal cell concentration from the starting inoculum.

Ten patients (eight women) were enrolled into this study and had the following demographic characteristics: mean age, 63 years (range 21–93 years); mean weight, 77 kg (range 50–148 kg); mean APACHE II (Acute Physiology and Chronic Health Evaluation II) score, 13 (range 4–24); and mean albumin, 2.4 mg/dL (range 1.5–4 mg/dL). Anidulafungin peak and trough serum levels were 4.9±2.4 mg/dL (range 1.5–4 mg/dL). Anidulafungin peak and trough levels of anidulafungin were similar to that of serum without drug. Anidulafungin was less active against C. parapsilosis isolates. Against C. parapsilosis strains with MICs of 0.25 and 0.5 mg/L, peak levels of anidulafungin were observed at the 6 and 24 h time periods, but regrowth occurred at 24 h (Figure 1). Against C. glabrata strains with MICs of 1.0 mg/L, inhibitory activity with anidulafungin was similar to that of serum without drug. Anidulafungin was less active against C. parapsilosis isolates. Against C. parapsilosis strains with MICs of 0.5 mg/L, no inhibitory activity was observed. Fungicidal activity was only found for Candida spp. (including C. albicans, C. glabrata and C. krusei) with anidulafungin MICs of <0.1 mg/L. This occurred by 6 h without subsequent regrowth.

The echinocandins inhibit the synthesis of 1,3-β-D-glucan in the fungal cell wall and anidulafungin is highly active in vitro against common Candida spp. (MIC90 = 0.12 mg/L) with the exception of C. parapsilosis (MIC90 = 2 mg/L).5

These agents exhibit concentration-dependent killing, and the Cmax/MIC ratio (4× the MIC) is strongly predictive of treatment success.5 Anidulafungin is highly protein bound (99% by equilibrium dialysis) and peak serum levels of free drug would be <0.1 mg/L at a dose of 100 mg/day.6 In our ex vivo study of peak serum levels from patients receiving anidulafungin, we observed fungicidal activity only against Candida spp. with MICs of <0.1 mg/L. No inhibitory or killing activity was observed against isolates at or near the current susceptible breakpoint of 2 mg/L.

In contrast to PD determinations, no clear correlation between MIC and clinical outcomes has been observed for specific Candida spp. when treated with anidulafungin.7 It appears from PD models and clinical trials with the echinocandins that host immune response and source control (e.g. catheter removal) must also be major determinants in clinical outcomes of Candida infections. Given that neither direct killing activity nor clinical efficacy by anidulafungin is captured by standard in vitro testing of Candida spp., susceptibility reports are unlikely to be helpful in the management of candidiasis.7

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Transparency declarations
None to declare.

References


