Correspondence

Adding 2% glucose to culture media does not influence the activity of caspofungin against Candida species

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Sir,

The activity of antifungal agents in vitro may vary according to procedural variations such as culture media, addition of supplements, incubation duration and endpoint determinations. The appropriate methods to assess the activity in vitro of the new candin antifungals have not been clearly defined. We assessed the influence of the addition of supplemental 2% glucose (2%G) to the culture medium on the activity of caspofungin in vitro against clinically significant Candida isolates.

A total of 50 isolates of Candida species recovered between 1996 and 2000 from blood cultures of cancer patients treated at Hôpital Maisonneuve-Rosemont, Montréal, Québec, Canada (HMR) were selected for testing. Antifungal susceptibility testing was carried out by a broth microdilution method according to the procedures described by the NCCLS. Standard caspofungin antifungal powder was supplied by Merck & Co. (Whitehouse Station, NJ, USA). Stock solutions were prepared in water. Serial two-fold dilutions were made in 2%G supplemented and non-supplemented RPMI 1640 medium (Gibco-BRL) buffered to pH 7.0 with 0.165 M MOPS buffer (Sigma) and 2%G supplemented and non-supplemented antibiotic medium 3 (AM3) (Difco Laboratories, Detroit, MI, USA). The final concentration of the solvent did not exceed 1% in any of the wells, with the final concentrations of caspofungin ranging from 0.006 to 64 mg/L. Drug-free and yeast-free controls were included. The trays were incubated in air at 35°C and MIC endpoints were read after 48 h of incubation. Following incubation, the trays were visually examined and the growth in each well was compared with that of the control (drug-free) well. The MICs were measured as the lowest concentration that resulted in a prominent decrease in turbidity compared with that of growth-control wells, using the turbidity numerical score proposed by the NCCLS. Candida parapsilosis ATCC 22019, Candida krusei ATCC 6258 and a fluconazole-resistant Candida albicans were used for quality control.

The effect of supplemental 2%G on RPMI-MOPS and AM3 after various incubation periods is shown in Table 1. Addition of 2%G to either RPMI-MOPS or AM3 had little effect on the observed MICs. Similarly, the length of incubation did not significantly influence the geometric mean MICs. After 48 h of incubation, MIC values were one- to two-fold higher than the 24 h values. Caspofungin, however, appears to be more potent in AM3, with geometric mean MICs four- to seven-fold lower in AM3 than in RPMI-MOPS. Test conditions have been found to have significant effects on the activity in vitro ofazole and triazole antifungals. However, with the candin antifungals, the impact of test conditions is unclear. Krishnarao & Galgiani recently analysed the test conditions on the activities in vitro of caspofungin and LY303366 against yeast isolates and failed to identify any significant impact. In contrast, several authors showed that echinocandins LY303366 and cilofungin appear to be considerably more potent against yeasts when tested in AM3 compared with RPMI-MOPS. In the present study, we have made similar observations, with caspofungin showing an increased activity in AM3 when compared with RPMI-MOPS. However, the discrepancies between study results might have been caused by the testing systems used in the different studies; like Pfaller et al., we used a broth microdilution system, whereas in their study, Krishnarao & Galgiani used a broth macrodilution system.

The lack of influence of glucose supplementation on the anti-Candida activity of caspofungin observed in our study was surprising. Glucose supplementation has been shown to prolong the exponential growth cycle and promote denser growth rates of Candida species. As a consequence, glucose supplementation of test medium has been proposed to facilitate and accelerate endpoint determinations after 24 h of incubation with fungistatic azoles. In our study, ambiguous endpoint determinations after 24 h of incubation were encountered on several occasions and were largely observed with C. parapsilosis. The intrinsic lower growth rate of this particular Candida species, which remains unaffected by glucose supplementation, was likely to be responsible for those ambiguous readings in our study. The neutral impact on the activity of caspofungin against the other Candida species tested in our study, despite presumably denser growth rates in glucose-supplemented medium, may be attributable to the rapid fungicidal activity of caspofungin against actively growing yeasts.
In conclusion, AM3 appears to increase the activity in vitro of caspofungin against clinical Candida isolates. Addition of 2%G to either RPMI-MOPS or AM3 had little impact on the observed MICs, and in general the MICs observed after 48 h of incubation did not vary from those obtained after 24 h of incubation.

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References


