Characterization of the inhibitory effect of voriconazole on the fungicidal activity of amphotericin B against Candida albicans in an in vitro kinetic model

Anders Lignell*, Elisabeth Löwdin, Otto Cars and Jan Sjölin

Section of Infectious Diseases, Department of Medical Sciences, Uppsala University Hospital, Uppsala, Sweden

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Objectives: The aim of the present investigation was to study and characterize the effect of voriconazole on the fungicidal activity of amphotericin B.

Methods: Four strains of Candida albicans susceptible to voriconazole were exposed to voriconazole and amphotericin B, either alone, simultaneously or sequentially in an in vitro kinetic model. Bolus doses resulting in voriconazole and amphotericin B concentrations of 0.005–5 and 2.5 mg/L, respectively, were administered. Antifungal-containing RPMI 1640 was eliminated and replaced by a fresh medium using a peristaltic pump, with a flow rate adjusted to obtain the desired half-lives. With two drugs tested, a computer-controlled dosing pump compensated for differences in the elimination rates. Using static time–kill methodology, one C. albicans strain was exposed to 5 mg/L voriconazole for varying durations followed by 2.5 mg/L amphotericin B after three repeated washes of voriconazole.

Results: Voriconazole and amphotericin B treatment alone resulted in fungistatic and fungicidal activities, respectively. Simultaneous administration of voriconazole and amphotericin B resulted in fungicidal activity, whereas only fungistatic activity was observed when repeated doses of amphotericin B were administered sequentially after voriconazole at 24–96 h. The inhibition of the fungicidal activity of amphotericin B was voriconazole dose-dependent, but seemed to be recovered once the voriconazole concentration fell below the MIC. The fungicidal activity was quickly regained after the removal of voriconazole, irrespective of the duration of voriconazole pre-exposure.

Conclusions: Voriconazole inhibited the fungicidal effect of sequentially administered amphotericin B in a concentration- and time-dependent manner; the clinical significance of this needs further investigation.

Keywords: antagonism, interaction, pharmacodynamics

Introduction

Combination antifungal therapy may be one method to improve outcome in invasive Candida infections. As animal model pharmacodynamic studies may have difficulties in simulating human pharmacokinetics, in vitro pharmacodynamic studies with simulated human pharmacokinetics represent an additional tool in the study of antifungal combinations, in that they can yield data on drug-specific antifungal activities that will be of interest before initiating clinical trials.

In a previous study, a new in vitro kinetic model for the study of combination treatment with drugs having different elimination rates was presented and validated.1 The model was used in a pilot experiment to demonstrate the antagonistic effect of voriconazole on the fungicidal activity of two sequential doses of amphotericin B against one strain of Candida albicans.

The aim of the present study was to further characterize this pharmacodynamic interaction in terms of: (i) effect on other C. albicans strains; (ii) duration of the voriconazole effect on amphotericin B using sequential dosing; (iii) effect of varying doses of voriconazole; and (iv) effect of varying voriconazole pre-exposure.

Materials and methods

Fungal strains

C. albicans CCUG 32723 (Culture Collection, University of Göteborg, Sweden) and three C. albicans strains isolated from...
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Patients’ blood, B415-1021, B424-6024 and B411-5022 (Department of Clinical Microbiology, Uppsala University Hospital, Uppsala, Sweden), were used. A suspension of C. albicans was prepared from a 24–48 h culture, from which one to two colonies were transferred into test tubes with 4 mL of RPMI 1640 (Invitrogen AB, Täby, Sweden) placed on a shaker and cultured at 35°C for 6 h, resulting in a standardized concentration of $\sim 10^7$ cfu/mL determined by plating on Sabouraud-dextrose agar plates and colony counting as described below.

**MIC determination**

MICs were determined in duplicate using CLSI (formerly NCCLS) methodology for broth dilution antifungal susceptibility testing of yeasts. The susceptibility endpoint for voriconazole was defined as the lowest concentration of the drug that resulted in an 80% reduction in visible growth. The MIC of amphotericin B was defined as the lowest concentration that resulted in the total inhibition of visible growth.

**Medium**

Sterile RPMI 1640 buffered to a pH of 7.0 with 0.165 M MOPS was used as the growth medium.

**Antifungal agents**

Voriconazole (Pfizer AB, Täby, Sweden) and amphotericin B deoxycholate (Sigma-Aldrich AB, Stockholm, Sweden) were obtained as sterile powders. Voriconazole and amphotericin B were dissolved in 1 mL of dimethyl sulphoxide (Sigma-Aldrich AB) and diluted in RPMI 1640 to obtain the desired concentrations. The antifungal solutions were prepared immediately before each experiment.

**In vitro kinetic model**

A previously described one-compartment *in vitro* kinetic model allowing exposure of *C. albicans* to simulated human serum pharmacokinetics for voriconazole and amphotericin B was used. The culture vessel (Bellco Glass Inc., Vineland, NJ, USA) with a total volume of 115 mL contained a magnetic stir bar for continuous mixing and had one arm at the side with a silicone membrane for sampling and injection of the studied drugs and the other connected by thin plastic tubing to a vessel for waste. Fresh medium was supplied to the culture vessel with a peristaltic pump (Watson–Marlow Allita 403U/VM4, Watson–Marlow Allita, Stockholm, Sweden) via latex tubing connected to the culture vessel.

The flow rate was adjusted to obtain the desired half-life of the antifungal agent. In regimens in which two agents with different elimination rates were used, a computer-controlled dosing pump (Harvard dosing-pump 55-2222, Harvard Apparatus Inc., Holliston, MA, USA) was used to infuse the agent with the longer half-life through a syringe connected via a plastic tubing to the culture vessel. This dosing pump also made it possible to compensate for the spontaneous degradation of the antifungals that may occur during the experimental conditions. The computer software (ARUComb 1.5, Snowfall Communications, Uppsala, Sweden) was used for programming the pump to infuse the drug at an exponentially decreasing rate. The *in vitro* model was placed in a thermostatic room at 35°C during the experiments. The equipment was sterilized by autoclaving between each experiment.

**Pharmacokinetic analysis**

The antifungal agents were eliminated at a constant rate according to the first-order kinetics $C = C_0 \times e^{-kt}$, where $C_0$ is the initial concentration, $C$ the concentration at time $t$, $k$ the rate of elimination and $t$ the time that has elapsed since the administration of the drug. Constant $k$ is similar to $F/V$, where $F$ is the dilution flow rate and $V$ is the volume of the compartment. Using this equation, the flow rate required to obtain a certain half-life could be calculated.

In the present experiments, the flow rate of the peristaltic pump was adjusted to obtain the desired half-life of 6 h for voriconazole. It has previously been described that amphotericin B degrades spontaneously in RPMI 1640, resulting in shorter half-lives than expected. To achieve a half-life at β-phase of 7 h for amphotericin B and to compensate for the spontaneous degradation, the computer-controlled dosing pump was prepared with an amphotericin B suspension concentration of 200 mg/L that was infused during the experiments.

To verify the pharmacokinetics of the model, antifungal concentrations were analysed in samples from regimens iv and v as described below: voriconazole and amphotericin B administered simultaneously at 0 h and voriconazole administered at 0 h followed by repeated administration of amphotericin B at 24, 48 and 72 h with RPMI 1640 starting concentrations of voriconazole and amphotericin B of 5 and 2.5 mg/L, respectively. Repeated sampling of 1 mL for the concentration analysis was made for every 24 h period at 0, 6, 12 and 24 h. Samples were stored frozen at −70°C until analysis. The regimens were performed in triplicate. The analysis of voriconazole and amphotericin B was performed using an HPLC-UV method developed at the Department of Clinical Pharmacology, Karolinska University Hospital, Huddinge, Sweden. The method showed good sensitivity with a lower limit of detection of 0.01 mg/L for both voriconazole and amphotericin B. Samples (0.2 mL) containing RPMI 1640, C. albicans, voriconazole and/or amphotericin B were adjusted to a pH of 3 with 50 μL of eluent solution containing acetonitrile (45%), ortho-phosphoric acid (0.2%) and ammonia (0.2%) and separated on a reverse-phase column [3 μm particle size, 4.6 mm internal diameter, 75 mm long; Phenomenex Luna C18(2); Skandavisska Genetic, Västra Förrunda, Sweden], with a mobile phase of acetonitrile (45%), ortho-phosphoric acid (0.2%) and ammonia (0.2%) delivered at a rate of 1.0 mL/min. The concentration of voriconazole was detected at a UV absorbance of 258 nm and that of amphotericin B at 406 nm. The standard curve concentration ranges were 0.2–8.1 mg/L for voriconazole and 0.2–8.0 mg/L for amphotericin B. Four-point standard curves, including a blank sample in RPMI 1640, were linear with slopes of 0.993 and 0.997 and $R^2$ values of >0.999 for voriconazole and amphotericin B in the order given. Mean coefficients of variation were 3.4% (voriconazole) and 5.7% (amphotericin B) in the concentration range of 0.2–6.0 mg/L.

**Killing experiments with a simulated kinetic profile**

Different antifungal regimens were simulated in the *in vitro* kinetic model. These included: (i) the control (no drug); (ii) voriconazole at 0 h; (iii) amphotericin B at 0 h; (iv) voriconazole + amphotericin B at 0 h and (v) voriconazole at 0 h and sequential doses of amphotericin B at 24, 48 and 72 h. Regimens i–v were performed with the use of *C. albicans* strains CCUG 32723, B415-1021, B424-6024 and B411-5022 with a starting concentration of voriconazole and amphotericin B of 5 and 2.5 mg/L, respectively. Regimen v was furthermore performed with the use of *C. albicans* strain CCUG 32723 and varying starting concentrations of voriconazole of 0.005, 0.05, 0.5 and 5 mg/L and a starting concentration of amphotericin B of...
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2.5 mg/L. Each regimen with the exception of the three blood isolates was tested in triplicate.

Serum protein binding has been reported to be 60% for voriconazole and 90% for amphoterin B. In the present experiments, the free concentration of voriconazole was simulated with initial concentrations spanning from a low level of around 1 × MIC for C. albicans CCUG 32723 to a high drug level in the range of those observed as peak concentrations in plasma after clinically relevant doses. The free concentration was also simulated for amphoterin B. The minimum fungicidal concentration (MFC) of amphoterin B for C. albicans CCUG 32723 tested in triplicate in the kinetic model was 0.5 mg/L. An MFC value twice the MIC for C. albicans is consistent with the results in other studies. However, as the aim was to demonstrate an inhibition of the fungicidal effect of amphoterin B and that this activity increases at higher concentrations, the amphoterin B level was somewhat higher than that found in clinical practice. The concentration chosen was also similar to that used by others in previous in vitro experiments.

A volume of 0.1 mL of C. albicans suspension was added to 110 mL of RPMI 1640 through one sidearm to yield a starting inoculum of ~10^7 cfu/mL. Volumes of 0.25–0.5 mL of voriconazole and/or amphoterin B solutions were added through one sidearm to yield the desired starting concentration. Repeated sampling before and after each antifungal dose at 0, 1.5, 3, 6, 12 and 24 h for viable counting was performed through the silicone membrane. The samples were serially diluted 10-fold in PBS. At least three samples (10 or 100 μL) from the original fungal suspension and/or dilutions were subsequently spread on Sabouraud-dextrose agar plates, incubated at 35°C and counted after 24–48 h. To avoid antifungal carryover, all samples were placed on the same spot on the Sabouraud-dextrose agar plates and allowed to diffuse into the agar for 3–5 min before spreading. If there was a clear zone where the sample had been placed after growth, the agar plate was divided into sections and the clear section was excluded from counting. To study the effect of carryover with this method, undiluted and 100-fold diluted samples containing 2.5 and 5 mg/L of amphoterin B and voriconazole, respectively, were spread on Sabouraud-dextrose agar plates. This resulted in negligible carryover with a mean log_{10} cfu/mL that was 1.5% and 1% higher in the diluted samples for amphoterin B and voriconazole, respectively. The limit of detection of the viable counts was 10 cfu/mL. Fungicidal and fungistatic effects were defined as a reduction in cfu/mL from the starting inoculum of ≥99.9% and <99.9%, respectively.

Time–kill experiments studying the effect of varying voriconazole pre-exposure

A C. albicans suspension with a concentration of ~10^7 cfu/mL was diluted 1:10. Aliquots of 0.1 mL of the diluted fungal suspension and 0.1 mL of voriconazole solution of 500 mg/L were subsequently added to test tubes containing 9.8 mL RPMI 1640, resulting in a C. albicans concentration of 10^5 cfu/mL and a voriconazole concentration of 5 mg/L. The test tubes were placed on a shaker in a thermostatic room at 35°C for 1, 8, 24 and 48 h and then washed three times by centrifugation at 1400 g for 10 min. The fungal pellet was resuspended in RPMI 1640 after each centrifugation. After washing, 9.9 mL of RPMI 1640 and 0.1 mL of amphoterin B solution were added to yield a final amphoterin B concentration of 2.5 mg/L. The test tubes were again placed on a shaker and incubated at 35°C for 24 h. Repeated sampling for viable counting was performed, and samples were processed as described earlier. Each regimen was performed in triplicate, and for each regimen, there was one control without antifungals containing C. albicans and RPMI 1640 only.

Statistics and calculations

Log_{10} cfu counts that were due to fungal growth or killing approximated to normal distribution. Fungal killing was defined as the difference in log_{10} cfu/mL before and 6 h after administration of amphoterin B. In the killing experiments in which C. albicans CCUG 32723 was exposed to a starting concentration of 5 mg/L voriconazole followed by repeated amphoterin B doses, fungal killing after the different amphoterin B doses was compared using one-way analysis of variance (ANOVA). In the killing experiments to study the result of varying voriconazole concentrations on the fungicidal effect of subsequent amphoterin B administration, a repeated-measures ANOVA was used to compare the effects of voriconazole concentration and time point for amphoterin B administration. Statistica 7.1 (StatSoft, Inc., Tulsa, OK, USA) was used in the statistical calculations.

Results

MIC determination

The MICs of voriconazole for C. albicans strains CCUG 32723, B415-1021, B424-6024 and B411-5022 were 0.004, 0.008, 0.016 and 0.008 mg/L, respectively. The MICs of amphoterin B for all strains were 0.25 mg/L.

Pharmacokinetic analysis

Maximum concentrations, minimum concentrations and half-lives of voriconazole and amphoterin B are shown in Table 1. Actual pharmacokinetic parameters were close to target values.

Antifungal effect of voriconazole and amphoterin B on different strains of C. albicans and duration of the inhibitory effect of voriconazole on amphoterin B

Time–kill curves for regimens in which C. albicans strains CCUG 32723, B415-1021, B424-6024 and B411-5022 were exposed to voriconazole and amphoterin B with starting concentrations of 5 and 2.5 mg/L, respectively, or combinations thereof are shown in Figure 1. For the unexposed control, there was an approximately 3 log_{10} unit increase in cfu/mL, demonstrating viability of the C. albicans strains and lack of growth-inhibiting substances in the medium. For all C. albicans strains, administration of voriconazole resulted in a fungistatic activity, whereas the administration of amphoterin B demonstrated a fungicidal effect. When voriconazole was combined with amphoterin B and administered simultaneously, the concentration of C. albicans was below the detection limit at 3 h, which did not differ from the rapid fungicidal effect of amphoterin B alone. In contrast, when amphoterin B was administered sequentially after voriconazole exposure, the fungicidal activity was abolished in all strains. In the experiments with C. albicans CCUG 32723, no significant fungal killing could be demonstrated after the amphoterin B exposures at 24, 48 and 72 h. After amphoterin B exposure at
72 h, a slight reduction in log₁₀ cfu/mL was demonstrated, which, however, was not statistically significant (P = 0.37). In the experiments with C. albicans strains B415-1021, B424-6024 and B411-5022, similar results were obtained at amphotericin B exposures after 24 and 48 h, whereas after 72 h, there was a slight reduction in the B415-1021 and B424-6024 strains that, however, did not meet the criteria of a fungicidal effect.

Effect of varying voriconazole doses

Amphotericin B-induced fungal killing of C. albicans CCUG 32723 at different time points after exposure to varying initial concentrations of voriconazole ranging from 0.005 to 5 mg/L is shown in Figure 2.

There was a significantly dose-dependent voriconazole-induced inhibition of the fungicidal activity of amphotericin B (P < 0.001). Furthermore, amphotericin B-induced fungal killing significantly changed over time (P < 0.001), with a slight increase after the highest voriconazole dose and a moderate decrease after the lower voriconazole doses, resulting in a highly significant dose by time interaction (P < 0.001).

Effect of varying durations of voriconazole pre-exposure

Time–kill curves demonstrating the effect of a constant concentration of amphotericin B on the C. albicans strain CCUG 32723, pre-exposed to 5 mg/L voriconazole for varying

Table 1. Experimental antifungal pharmacokinetic values

<table>
<thead>
<tr>
<th>Parameter</th>
<th>VRCₘₐₓ (mg/L)</th>
<th>AMBₘₐₓ (mg/L)</th>
<th>VRC₀ h (mg/L)</th>
<th>AMB₂₄ h (mg/L)</th>
<th>AMB₄₈ h (mg/L)</th>
<th>AMB₇₂ h (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cₘₐₓ (mg/L)</td>
<td>4.7 ± 0.2</td>
<td>1.9 ± 0.3</td>
<td>4.6 ± 0.0</td>
<td>2.4 ± 0.2</td>
<td>2.2 ± 0.4</td>
<td>2.9 ± 0.1</td>
</tr>
<tr>
<td>Cₘᵦₐₙ (mg/L)</td>
<td>0.3 ± 0.0</td>
<td>0.1 ± 0.0</td>
<td>0.4 ± 0.0</td>
<td>0.1 ± 0.0</td>
<td>0.1 ± 0.0</td>
<td>0.3 ± 0.0</td>
</tr>
<tr>
<td>t₁/₂ (h)</td>
<td>6.2 ± 0.0</td>
<td>6.0 ± 0.3</td>
<td>6.6 ± 0.2</td>
<td>5.3 ± 0.2</td>
<td>5.4 ± 0.5</td>
<td>7.5 ± 1.7</td>
</tr>
</tbody>
</table>

VRC, voriconazole; AMB, amphotericin B; simult, simultaneous administration of VRC and AMB; 0–72 h, time point for administration of VRC and AMB. Values reported are means ± SE of three experiments. Antifungal concentrations correspond to the free non-protein bound fraction.

Figure 1. Time–kill plots of voriconazole (VRC) and amphotericin B (AMB) regimens against C. albicans strains CCUG 32723 (a), B415-1021 (b), B424-6024 (c) and B411-5022 (d) in the in vitro kinetic model. Filled diamonds, growth control; filled squares, VRC administered at 0 h; filled triangles, AMB administered at 0 h; open squares, simultaneous administration of VRC and AMB at 0 h; open triangles, VRC administered at 0 h followed by AMB at 24, 48 and 72 h and broken line, limit of detection. In (a), the values plotted are means ± SD of three experiments.

Figure 2. Fungal killing of C. albicans CCUG 32723 after amphotericin B administration at different time points after exposure to varying doses of voriconazole at 0 h, resulting in different initial peak concentrations of 0.005 (black bars), 0.05 (dark grey bars), 0.5 (light grey bars) and 5 mg/L (white bars). Each bar represents the mean ± SE of three experiments.
in the absence of voriconazole, an early fungicidal activity of amphotericin B was detected. The killing rate was not significantly reduced after longer pre-exposure, and even after a pre-exposure of 48 h, the time for reducing the fungal count by $3 \log_{10}$ was only 2 h.

**Discussion**

In this study, it was demonstrated in all four strains of *C. albicans* tested that administration of voriconazole inhibited the fungicidal activity of sequentially administered doses of amphotericin B. Thus, this negative interaction appears valid for *C. albicans* isolates susceptible to both voriconazole and amphotericin B. The fungicidal activity of amphotericin B did not return against any of the *C. albicans* strains during the experiments after exposure to high, but clinically relevant, doses of voriconazole. At 72 h, when the voriconazole concentration had reached levels near or somewhat below the MIC, there was some reduction in colony counts, but the effect of the amphotericin B dose did not meet the criterion of a fungicidal effect. The highest amphotericin B killing rate after the third dose was found against the strain B424-6024 (Figure 1c), which demonstrated an MIC of voriconazole that was slightly higher than those for the other strains.

This result is in agreement with that from the experiments in which different initial voriconazole concentrations were employed. In these experiments, the negative interaction between voriconazole and amphotericin B was shown to be voriconazole concentration-dependent. The lowest voriconazole dose with a starting concentration of the magnitude of that of the MIC did not inhibit the fungicidal activity of an amphotericin B dose after 24 h, whereas the higher voriconazole doses significantly reduced the amphotericin B-induced killing.

The results of these experiments indicate that when the concentration of voriconazole falls below the MIC, the fungicidal effect of amphotericin B is gradually regained. The absence of long persisting antagonistic effects once the concentration has fallen below the MIC is further supported by the experiments in which voriconazole after varying durations was washed away. The fungicidal amphotericin B effect was retrieved within a few hours, despite exposures to high concentrations of voriconazole for up to 48 h. This finding is, to some extent, in contrast to what was found in the *in vitro* studies by Louie et al., in which longer pre-exposures to fluconazole resulted in prolonged inhibition of the fungicidal activity of amphotericin B. It is difficult to explain these diverging results, but it may be speculated that there are differences in the affinities to the 14-α-sterolmethylase or to efflux pumps between voriconazole and fluconazole. This conjecture, however, needs further investigation.

There was also a significant effect of time. In contrast to the higher voriconazole doses at which the inhibitory effect on amphotericin B decreased by time, leading to increased killing, the lower doses demonstrated decreased amphotericin B killing by time, resulting in a highly significant concentration by time interaction. The time effect of the higher voriconazole doses is probably caused by a reduced concentration effect, whereas the decreased amphotericin B killing at the lower voriconazole doses is more difficult to explain. It seems unlikely that this phenomenon is caused by voriconazole. More likely, there is an adaptive resistance against amphotericin B due to repeated amphotericin B administration, but this needs further investigation. Adaptive resistance has previously been reported to be induced by aminoglycosides and β-lactam antibiotics, both *in vitro* and in animal models, but has, to the best of our knowledge, not been described in antifungal agents.

To what extent these results can be extrapolated to animal experiments and clinical practice is not yet known. During the past decades, there has been an intense discussion about the clinical relevance of similar findings regarding other azoles and amphotericin B. There is a theoretical concern thatazole agents negatively influence the activity of amphotericin B because there will be less ergosterol available for the polyene to bind to as a result of the azole-inhibited ergosterol synthesis. A number of *in vitro* studies, animal models and clinical reports on this matter have been reviewed elsewhere with mixed results. In the majority of the *in vitro* studies, antagonism has been reported. In some of the studies that failed to identify antagonism between azoles and amphotericin B, the drugs were administered simultaneously. Such a finding is in agreement with our results and those from others investigating strains in which antagonism after sequential treatment can be demonstrated. The mechanism for this is probably that there is no time for theazole to affect the receptor before the fungal cell is killed by the action of amphotericin B. Using an *in vitro* kinetic model, Lewis et al. reported antagonism when fluconazole was administered before amphotericin B. This effect was later observed in a rabbit model by Louie et al., in which amphotericin B administered after treatment with fluconazole resulted in a slower clearance of fungi from infected tissues than after treatment with amphotericin B alone. In one clinical trial, often referred to in this context, by Rex et al., fluconazole and amphotericin B in combination resulted in a clear trend towards a better outcome than fluconazole treatment alone. However, in this study, a comparison was not made between the combination treatment and amphotericin B alone, which would have been of special interest with reference to the experimentally demonstrated antagonism. The finding that the azole–amphotericin B combination is superior to the azole alone is in good agreement.
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with our result and those of others, because the retained fungicidal effect of amphotericin B when combined with fluconazole right from the start leads to improved sterilization in comparison with that of the fungistatic effect of fluconazole alone. It has also been advocated that the patients in the study by Rex et al. who had been given fluconazole before enrolment, comprising about half of the study population, demonstrated almost the same result as those without prior fluconazole treatment. However, there was no information about the timing and the magnitude of the fluconazole doses given. Most probably, doses were low because exclusion criteria set a maximum dose of 800 mg having been received in the 14 days before study inclusion. These facts and the low number of patients would thus result in a high statistical β-error, indicating a considerable risk of not detecting an antagonism that actually exists.

Voriconazole has been considered one of the options for empirical antifungal therapy in patients with neutropenia and persistent fever. A fungicidal effect at the start of treatment in these vulnerable patients may be of importance, and if there is a need to change antifungal treatment from voriconazole to an amphotericin B preparation, it cannot, at present, be ruled out that the interaction demonstrated in this study might be of clinical relevance.

In summary, the present study demonstrated an antagonistic effect of voriconazole on the fungicidal activity of sequential doses of amphotericin B against strains of C. albicans, susceptible to both voriconazole and amphotericin B. With a high-dose regimen, voriconazole inhibited the fungicidal activity for up to 72 h. The antagonistic effect was shown to be voriconazole concentration-dependent, and amphotericin B activity was regained once the concentrations had fallen below the MIC values. The clinical relevance of these findings requires further investigation.

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Transparency declarations

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