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Assessment of antifungal drugs' activity against some *Candida albicans* isolates in the presence or absence of human albumin: a study employing an *in vitro* pharmacokinetics / pharmacodynamics model

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Abstract

Invasive candidiasis associated with the dissemination of endogenous *Candida* species is a fatal condition linked to high rates of morbidity and mortality. Progressive drug resistance necessitates the need for prompt and effective therapy. Therefore, choosing a specific and effective treatment is crucial. A two-compartment *in vitro* pharmacokinetics (PK) / pharmacodynamics (PD) model has been used for this purpose, and the PD behaviours of amphotericin B (AMB; at 2.5 and 5 mg/L), voriconazole (VOR; at 1.5 and 3 mg/L), and itraconazole (ITR; at 1.5 and 3 mg/L) were assessed against two *Candida albicans* isolates (a sensitive and resistant one; ATCC-90028 and ATCC-10231, respectively) with or without the addition of human albumin (2%). PK were simulated as time-concentration profiles, while the PD susceptibility of all drug doses has been assessed through the minimum inhibitory concentration (MIC), the relative optical density of fungal growth, and the exposure - effect relationship ($fAUC_{0-24}$/MIC). A fungicidal activity without the presence of albumin was seen against both isolates of *C. albicans* at the highest dose of VOR, while the addition of albumin potentiated the efficacies of AMB and of VOR against both isolates, with no effect for ITR. Finally, human albumin exerted a variable and dose-dependent effect on the activities of some antifungal agents.

KEYWORDS
antifungal drugs, *Candida* species, *in vitro* model, pharmacokinetics, pharmacodynamics

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1. INTRODUCTION

Invasive candidiasis remains a significant clinical cause of high morbidity and mortality, especially when associated with a multi-drug-resistant strain in the intensive care unit (ICU). *Candida albicans* is the most common species causing candidaemia, and accounts for 56% of all *Candida* infections in ICU patients [1]. Amphotericin B (AMB) is

still, for decades, the treatment of choice for invasive candidiasis. *C. albicans* has acquired resistance to azole due to overexpression with cross-resistance from non-pathogenic *C. albicans* [2]. Ultimately, the clinical significance of the antifungal activity of azole drugs is decreased. Moreover, many patients have low serum drug exposure after oral administration; therefore, intravenous formulations are preferred due to their less variable pharmacokinetics (PK) and for their higher exposure that is ideal for the treatment and prevention of azole-resistant candida infections [3,4]. An *in vitro* PK / pharmacodynamics (PD) model has been used in this study in order to explore the details and the differences among AMB, voriconazole (VOR), and itraconazole (ITR) against two *C. albicans* strains, in the presence and absence of albumin.

2. MATERIALS AND METHODS

Candida isolates: Two *C. albicans* isolates (a sensitive and a resistant one; ATCC-90028 and ATCC-10231, respectively) were used in order to simulate different time-concentration profiles. The minimum inhibitory concentration (MIC) values were 0.06–2 and 0.12–4 mg/L, respectively, based on the median (range) of Clinical and Laboratory Standards Institute (CLSI). The isolates were stored in normal sterile saline with 10% glycerol at -70°C, and were revived by subculturing on Sabouraud dextrose agar (SDA) plates (SGC2; bioMérieux, Marcy l'Etoile, France) so as to ensure purity and viability. Inoculum suspensions were prepared in normal sterile saline from 24-h cultures, and were adjusted to a final inoculum of 2×10^4 colony-forming units (CFU)/mL in the *in vitro* model by using a counting chamber.

Antifungal drugs and medium: AMB (50-mg vial; BioLab, India), VOR (200-mg vial; Zydus Cadila, India) and ITR (200-mg tablet; Taj Pharmaceuticals, India). The medium used in the *in vitro* PK/PD model was RPMI-1640, buffered to pH 7.0 (Sigma Aldrich, Darmstadt, Germany), and supplemented with 100 mg/L chloramphenicol (AppliChem GmbH, Darmstadt, Germany). Moreover, a 2% albumin (Kedrion biopharma; Italy) was added as required.

In vitro PK/PD model: A two-compartment PK/PD model consists of a 500-mL beaker glass containing fresh RPMI-1640 medium to an initial volume of 5 mL from floating tubes with a dialytic membrane (20 kDa) for each isolate of *C. albicans* and antifungal dosing regimen. The central one was connected to a peristaltic pump (Minipuls Evolution; Gilson Inc., Middleton, WI, USA), adding fresh medium in order to dilute its content at a rate equal to the clearance of antifungal drugs in human plasma [5,6] (Figure 1K).

In vitro PK: The simulated targeting free (unbound) maximum plasma concentrations (fC_{max}) of 2.5 and 5.0 mg/L of AMB, 1.5 and 3.0 mg/L of VOR, and 1.5 and 3.0 mg/L of ITR, as well as a half-life ($t_{1/2}$) of 12–24 h were assessed in order to better describe the exposure - effect relationship. The simulated time - concentration profiles were chosen in order to simulate different 24-h drug exposures observed.

In vitro PD: In order to estimate the fungal load inside the floating dialytic tubes for each antifungal dosing regimen, 200-µL samples were collected at regular intervals up to 24 h, and fungal growth was assessed spectrophotometrically by measuring the relative optical density (ROD) at 405 nm, at each dilution. The ROD_{405} for each drug concentration at a specific timepoint, in relation to the control growth at same timepoint as well as over time, was plotted.

In vitro PK/PD analysis: The PK/PD index as the $fAUC_{0–24}$/MIC ratio was calculated for each simulated dose and isolate during the experiment. The drug exposure - response relationship, expressed as 24-h growth reduction for each dosing regimen and isolate, was compared with values at the start of dosing that were analysed by a nonlinear regression analysis using a sigmoidal model with variable slope. All data were analysed using GraphPad Prism version 5.0 for Windows (GraphPad Software, San Diego, CA, USA).

3. RESULTS

As shown in Figure 1, the nonlinear inhibition represented the mean of ROD values for each C_{max} of antifungal agents against both isolates, in the presence and absence of 2% human albumin. A fungicidal effect of VOR without addition of albumin was seen only with a C_{max} (3 mg/L) on both isolates (Figures 1D and 1E), while a similar picture was seen under other C_{max} conditions of other antifungal agents, but followed with a regrowth of *Candida* (Figures 1A, 1B, 1G, and 1H). The addition of 2% of human albumin leads to a complete inhibition of growth of both isolates treated with AMB or VOR (Figures 1A–1E). No significant growth inhibition was seen with ITR under both C_{max} conditions (Figures 1G and 1H). The $AUC_{0–24}$/MIC represented the exposure-effect relationships as the AUCs were determined for each simulated dose, and any deviation from the target values was adjusted. The 24-h change in the ROD *versus* $fAUC_{0–24}$/MIC relationship for both *C. albicans* isolates is displayed as a sigmoidal curve (Figures 1C, 1F, and 1I) for all antifungal agents in relation to control (Figure 1J).

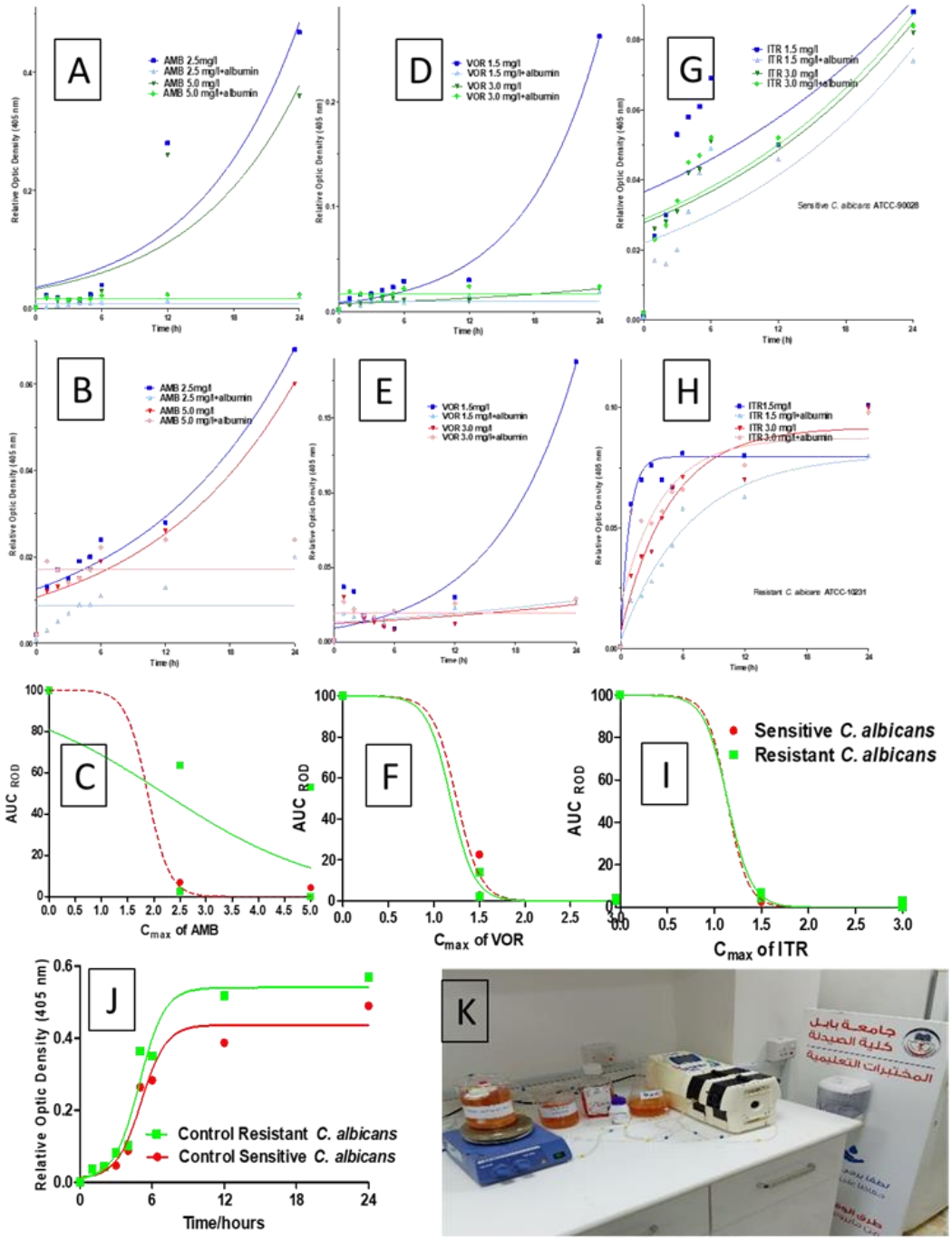

Figure 1. Data obtained from our *in vitro* pharmacokinetics / pharmacodynamics (PK/PD) model. Simulated human dosing with 2.5 and 5.0 mg/L of amphotericin B (AMB; **A** and **B**), 1.5 and 3.0 mg/L of voriconazole (VOR; **D** and **E**), or 1.5 and 3.0 mg/L of itraconazole (ITR; **G** and **H**) was used against two isolates of *C. albicans* (a sensitive and a resistant one; ATCC-90028 and ATCC-10231, respectively) in the presence and absence of 2% albumin for each maximum concentration (C_{max}). Panels **C**, **F**, and **I** represent the single-dose exposure-efficacy relationship of AMB, VOR, and ITR, respectively, against each isolate of *C. albicans*. Panel **J** presents the growth indices in control isolates, while panel **K** presents the *in vitro* PK/PD model used in our experiments.

4. DISCUSSION

Antifungal resistance is still a big challenge for physicians and researchers that needs to be addressed and managed effectively. Different antifungal agents that have been assessed by an *in vitro* PK/PD model have shown different drug efficacies. A fungicidal activity in the absence of albumin was observed against both isolates of *C. albicans* with the highest dose of VOR, and this finding might be due to the post-antifungal effects of the azole group [7]. The addition of albumin potentiates the efficacies of AMB and of VOR against both isolates, with no effect for ITR [8,10]. Finally, human albumin has a variable effect that is dose-dependent on the activities of some antifungal agents. Moreover, albumin itself may have a fungicidal activity against *C. albicans* [10].

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CONFLICT OF INTEREST STATEMENT
The authors declare no conflicts of interest.

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