In vitro evaluation of the efficacy of voriconazole-anidulafungin combination against azole-susceptible and -resistant *Aspergillus fumigatus* strains with a pharmacokinetic-pharmacodynamic model

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INTRODUCTION

Voriconazole has been adopted as the gold standard for the treatment of invasive aspergillosis. Nevertheless, the alarming increase in the frequency of azole-resistant *A. fumigatus* clinical isolates challenges current monotherapeutic strategies. Azoles are often combined with echinocandins since the two classes of antifungal compounds possess different mechanisms of action.

We therefore investigated the *in vitro* pharmacodynamics of the combination of voriconazole (VRC) and anidulafungin (ANID) against azole-susceptible and -resistant *A. fumigatus* isolates in a new *in vitro* PK-PD model.

MATERIALS AND METHODS

Isolates. Two clinical *A. fumigatus* isolates AFM8196 and AFM5235 with ANID CLSI MEC of 0.06 mg/L and CLSI VRC MIC of 0.12 mg/L (wild-type susceptible without *cyp51A* mutations) and 2 mg/L (resistant strain harboring the TR/L98H mutation), respectively, were studied.

***In vitro* PK-PD model.** A recently developed two compartment PK-PD dialysis/diffusion closed model was used (Meletiadis J, *AAC* 2012; 56: 403-10). The model has been adapted to include two drugs with different half-lives i.e. different flow rates for each drug enabling thus the study of drug combinations (**Figure 1**).

Pharmacokinetics. Human standard dosages of 4 mg/kg for VRC and 100 mg for ANID were simulated. In particular, the mean, lower and upper 95% confidence interval limits (CIL) of the free drug levels were calculated on the basis of VRC and ANID protein binding of 58% and 99%, respectively, and previously reported total maximum concentrations (C_{max}) in human serum (Purkins L. et al AAC 2002, Liu P. et al ACC 2013). Thus, time-concentration profile of VRC and ANID with fC_{max} of 1.5, 0.35, 3 mg/L and 0.08, 0.01, 0.16 mg/L and half-lives 6h and 24h, respectively, were simulated alone and in all 3x3 combinations. After inoculation of the IC with *Aspergillus* conidia (10^3 cfu/mL), VRC and ANID were added alone and in combination in both compartments every 12 and 24h, respectively, for 72h and incubated at 37°C. Drug levels were determined by microbiological diffusion assays.

Pharmacodynamics. Fungal growth in monotherapies and their combinations was assessed by monitoring the galactomannan (GM) production in the inoculated dialysis tubes using a commercially available sandwich enzyme-linked immunoassay. Pharmacodynamics were also assessed with real-time PCR. The reduction of the PCR conidial equivalents (CE) after 72h of incubation compared to 0h was calculated for each strain and simulated regimen.

Drug interaction analysis was performed according to Bliss independence analysis. All experiments were carried out in duplicate and were independently performed on two different days with individually prepared inocula.

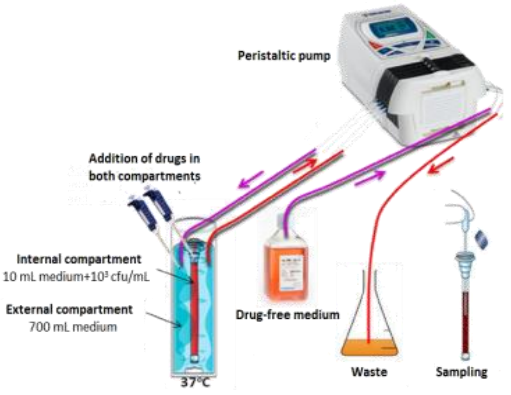

Figure 1. *In vitro* pharmacokinetic-pharmacodynamic model.

RESULTS

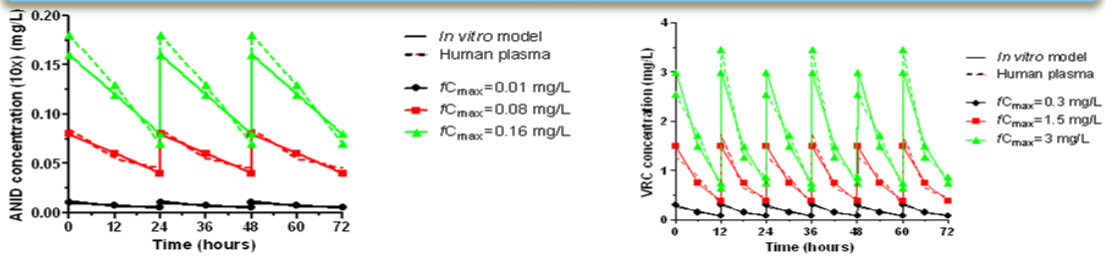

Figure 1. *In vitro* pharmacokinetics (solid lines) of VRC and ANID simulating free human plasma concentration (dashed lines).

- The *in vitro* simulated steady state drug pharmacokinetics were close to the target values observed in human plasma (**Figure 1**).
- ANID alone resulted in minimal (<5%) suppression of GM production for both isolates at all tested concentrations, whereas VRC alone suppressed GM production of AFM8196 by 81%, 100%, 100% and of AFM5235 by 7%, 32%, 38% at fC_{max} 0.35, 1.5 and 3 mg/L, respectively.
- The combination was independent at all dosing regimens except at the lowest fC_{max} of 0.35 mg/L of VRC and 0.01-0.08 mg/L of ANID where synergy was found for the susceptible AFM8196 (12%-14%) and the resistant AFM5235 (20-22%) isolate (**Figure 2**).
- In addition, PCR CE of monotherapies were reduced down to $3\log_{10}$ when the two drugs were combined confirming the enhanced killing of combination (**Figure 3**).

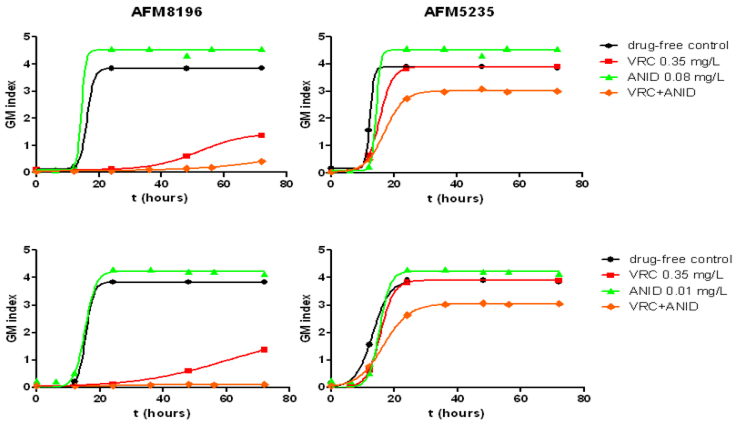

Figure 2. *In vitro* pharmacodynamics of VRC + ANID against a wild type VRC susceptible (left graphs) and a non-wild type VRC resistant harboring the *cyp51A* TR/L98H (right graphs) *A. fumigatus* isolate. The lines represent the regression lines obtained with the E_{max} model.

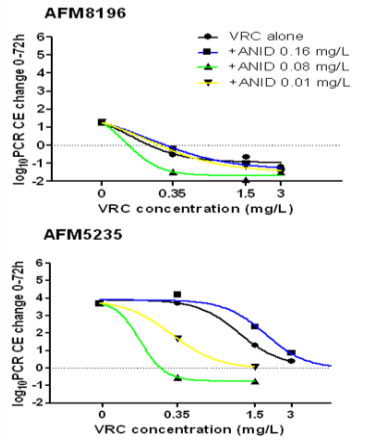

Figure 3. Real time PCR data of VRC + ANID. Changes in $\log_{10}$ PCR CE of VRC alone – in combination are presented for each combination and strain.

CONCLUSIONS

✓The combination of VRC plus ANID was Bliss independent for combinations with serum concentrations equal to or higher than the average levels observed in patients' serum.

✓Synergistic interactions were found at low VRC concentrations indicating that VRC+ANID may be beneficial for patients with subtherapeutic VRC serum concentrations.