Optimizing antifungal combination therapy of voriconazole+amphotericin B against *Aspergillus fumigatus* using a pharmacokinetic-pharmacodynamic model

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INTRODUCTION

Polyene-triazole combination therapy is often used for the treatment of refractory invasive aspergillosis. *In vitro* this combination demonstrated concentration dependent synergistic and antagonistic interactions raising questions on the role of therapeutic drug monitoring (TDM) for optimization of antifungal combination therapy.

We therefore investigated the pharmacodynamic effects of voriconazole (VRC) plus amphotericin B (AMB) combination against *A. fumigatus* isolates simulating human serum concentration-time profiles in a new *in vitro* PK-PD model and determined the serum concentrations that maximize the synergistic and minimize the antagonistic interactions.

MATERIALS AND METHODS

Isolate. The two-drug combination was tested against *A. fumigatus* strain NIH4215 (ATCC no. MYA-3626) susceptible to AMB and VRC with CLSI MICs 1 and 0.5 mg//L, respectively.

***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. VRC and conventional AMB serum concentration-time profiles were simulated with a half-life of 6h for VRC and 2h and 12h for the alpha and beta elimination phase, respectively, of AMB. In particular, twenty-one different combination regimens including monotherapies and drug-free control were investigated simulating various VRC and AMB fC_{maxs} (7.2, 3.4, 1.7, 0.8, 0.4 mg/L and 2.4, 0.6, 0.3, 0.1, 0.05, 0.025, 0.012 mg/L, respectively). After inoculating the internal compartment with *Aspergillus* conidia (10^3 cfu/mL), VRC and AMB 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. In order to estimate the antifungal effect of monotherapies and their combination, 200 μL from the inoculated dialysis tubes were sampled at regular intervals to determine galactomannan (GM) levels using a commercially available sandwich enzyme-linked immunoassay (Platelia Aspergillus, Biorad).

Analysis. PK data were subjected to nonlinear regression analysis based on the one- and two-compartment PK model for VRC and AMB, respectively, and the area under the concentration-time curve (AUC) was calculated. The % of fungal growth was calculated based on the area under the galactomannan index curve (AUC_{GM}) of each monotherapy and their combination divided by the AUC_{GM} of the drug-free control. 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.

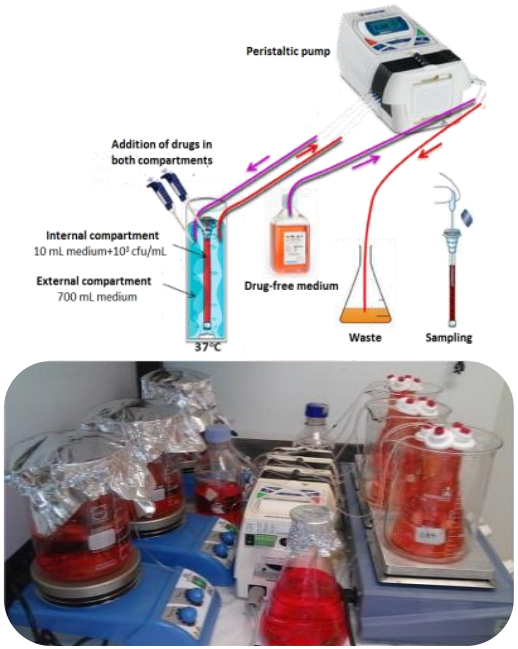

Figure 1. *In vitro* pharmacokinetic-pharmacodynamic model.

RESULTS

- The *in vitro* model simulated well steady state drug pharmacokinetics. In particular, the initial fC_{max} of both drugs were close to the target values (maximum deviation 18%), with average $t_{1/2}$ 5.5-6.7h for VRC and $t_{1/2,\alpha}$ 0.2-1h and $t_{1/2,\beta}$ 6-8h for AMB.
- After 72h of incubation both drugs completely inhibited the fungal growth except the lowest simulated fC_{max} i.e. 1.7-0.4 mg/L and 0.1-0.012 mg/L for VRC and AMB, respectively. The E_{max} model described the data well, as demonstrated by an R^2 value of >0.89 (**Figure 2**).

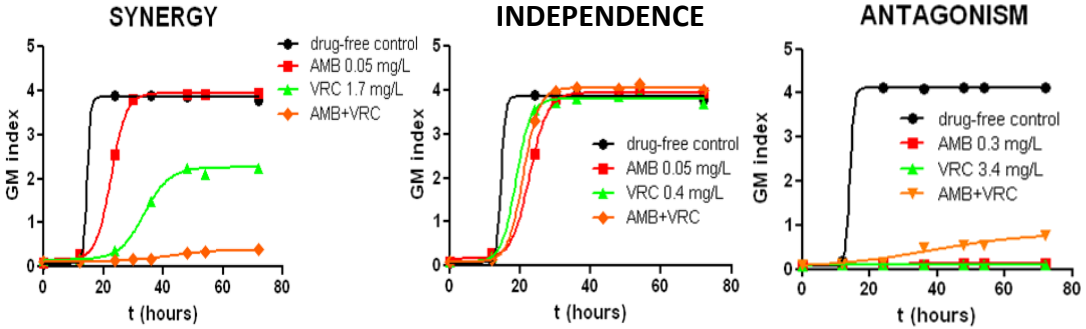

Figure 2. Representative *in vitro* pharmacodynamics of VRC plus AMB against *A. fumigatus*. The lines represent the regression lines obtained with the E_{max} model.

- Most combination regimens exerted independent effects (**Table 1,** yellow combinations). Synergy (24-34%) was observed for combinations with VRC fC_{max} 1.7 mg/L and AMB fCmaxs ≤0.1 mg/L. Combination regimens with VRC fC_{max} 0.8 mg/L and AMB fC_{max} 0.1 and 0.05 mg/L also exerted synergistic effects (19-31%), whereas antagonism (-33 - -29%) was found at lower AMB fC_{max} ($p<0.05$).
- Extrapolating to human dosages and taking into account the protein binding of VRC (58%) and AMB (95%), the fCmax where synergistic interactions were observed can be achieved in human plasma after standard dosing of VRC (4 mg/kg) and AMB (1 mg/kg) (Purkins L. et al ACC 2002, Ayestarán A. et al AAC 1996).

Table 1. *In vitro* drug interactions of VRC plus AMB combination.

VOR fC_{max} (mg/L)	AMB fC_{max} (mg/L)						
	0.01	0.02	0.05	0.1	0.3	0.6	2.4
7.2	ND	ND	ND	-7%	-8%	-7%	-4%
3.4	ND	ND	ND	-13%	-13%	-9%	-6%
1.7	24%	27%	34%	26%	-9%	-6%	-4%
0.8	-29%	-33%	31%	19%	ND	ND	ND
0.4	ND	ND	-8%	-7%	ND	ND	ND

Footnotes:

1. Green : Bliss synergy, yellow : Bliss independence, red: Bliss antagonism.
2. ND: not determined.
3. The blue frame represents the interactions at clinically achievable in serum free drug concentrations.

CONCLUSIONS

- ✓The double combination of voriconazole plus amphotericin B was synergistic against *A. fumigatus* at clinically achievable serum concentrations.
- ✓These concentrations may occur in patients with sub-therapeutic drug exposures.
- ✓Because antagonistic interactions were also observed, TDM of combination regimens could be employed in order to optimize antifungal combination therapy.