Determination of susceptibility breakpoints for posaconazole and *Aspergillus fumigatus using a* pharmacokinetic-pharmacodynamic model

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INTRODUCTION

Aspergillus fumigatus has become the most widespread opportunistic fungal pathogen, causing severe and commonly fatal invasive fungal infections in neutropenic hosts. Posaconazole is a triazole antifungal agent commonly used for prophylaxis and occasionally for treatment of invasive aspergillosis.

The emergence of azole-resistant isolates is of great concern since azole therapy against these isolates is often associated with clinical failure. Reliable susceptibility breakpoints for detecting these isolates are on high demand.

Therefore, we investigated the pharmacokinetic (PK) and pharmacodynamic (PD) properties of posaconazole using an *in vitro* model simulating human PK. For this purpose four clinical isolates of *A. fumigatus* with different *cyp51a* mutations and in vitro susceptibility to posaconazole were used. Human pharmacokinetics of posaconazole were simulated *in vitro* and the Monte Carlo analysis was applied for determination of susceptibility breakpoints.

MATERIALS/METHODS

Isolates: Four clinical *A. fumigatus* isolates with characterized azole resistance mechanisms and distinct susceptibility profiles to posaconazole were studied. The CLSI MICs were determined at 0.03, 0.5, 0.5 and >16 mg/L for AZN8196 (wild type), TR/L98H (V52-35), M220I (V28-77) and G54W (V59-73), respectively. Inocula were prepared in sterile saline with 1% Tween 20, in order to obtain a final concentration of 10^3 CFU/mL.

***In vitro* PK-PD model.** A previously described *in vitro* PK-PD diffusion model simulating human PKs was used in this study (Meletiadis J, *AAC* 2012; 56: 403-10) (**Figure 1**). The dose of 400mg bid, which corresponds to maximum blood concentration of posaconazole equal to 0.851 ± 0.69 mg/l (mean ± SD) and half-life of 12h was simulated (A. J. Ullmann, 2006.AAC., 50(2):658) .

Posaconazole was administered every 12h for 72h of total incubation, targeting total maximum concentrations of 0.15, 0.85 and 2.25 mg/l and half life of 12h (**Figure 2**). The concentration of posaconazole was assessed with a bioassay previously described (Rochat et al, AAC 2010) and the % growth was estimated based on the galactomannan production, measured with a sandwich enzyme-linked immunoassay (Platelia Aspergillus, Biorad) for each dose and isolate.

Analysis. The % of growth inhibition was calculated using the area under galactomannan index-72h time curve (PD parameter). The area under the concentration-12h time curve (AUC) was calculated as the PK parameter. The PK-PD relationship was analyzed with the nonlinear regression analysis using the E_{max} model and the AUC/MIC associated with 50% of maximal activity was estimated (PD target). 1000 patients were then simulated using Monte Carlo analysis and the % of patients attained the PD target was determined for different MICs.

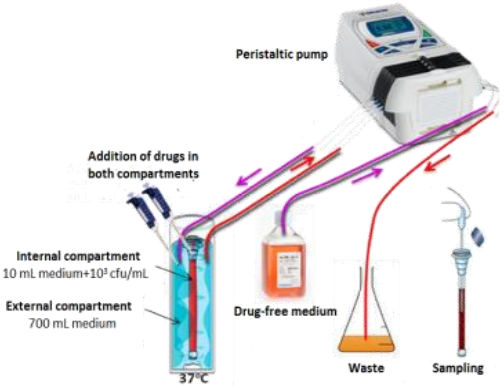

Figure 1. *in vitro* pharmacokinetic-pharmacodynamic model

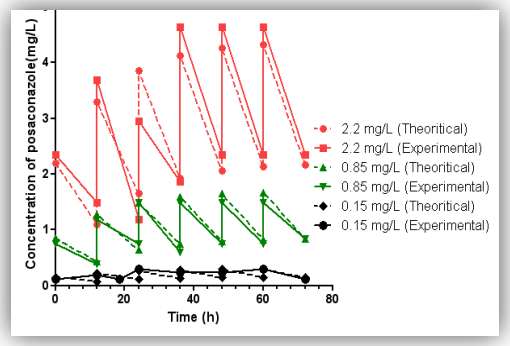

Figure 2. In vitro pharmacokinetic data

RESULTS

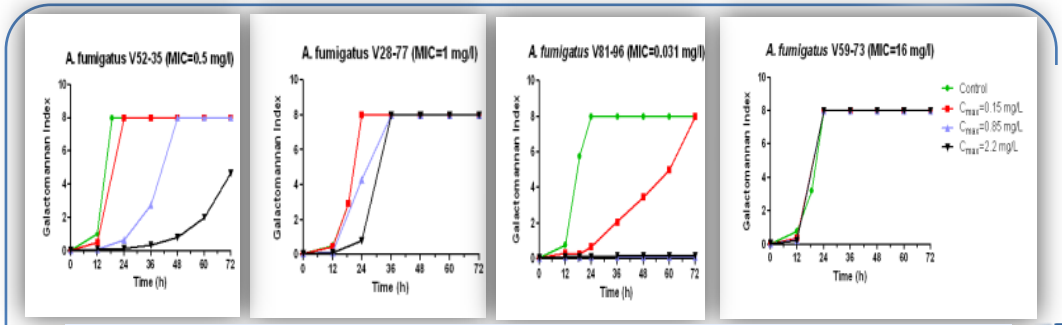

Figure 3. In vitro pharmacodynamic data. Galactomannan index-time curves of the four *A. fumigatus* isolates.

- Posaconazole demonstrated the maximum *in vitro* activity (>58% growth inhibition) against the wild-type isolate and the least activity (<5% growth inhibition) against the isolate harboring the G54W mutation (**Figure 3**).
- For the isolates with the same MIC, the *in vitro* activity of posaconazole was higher against the strain with the TR/L98H mutation compared to the strain with the mutation M220I (85% vs. 21% growth inhibition at 2.25 mg/L, respectively) (**Figure 3**).

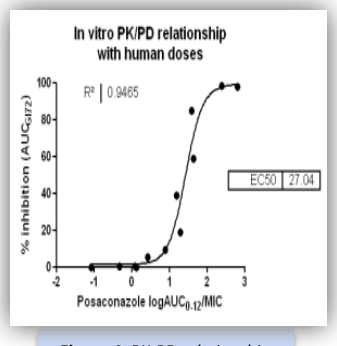

Figure 4. PK-PD relationship

- The PK-PD relationship followed a sigmoid curve (R^2=0.885) with an AUC/MIC corresponding to 50% of maximum efficacy of 27.04 (17.68-41.35) (**Figure 4**) .
- Monte Carlo analysis showed >70% and <30% target attainment rates for isolates with MICs ≤0.0625 mg/l and ≥0.5 mg/l, respectively (**Figure 5**).

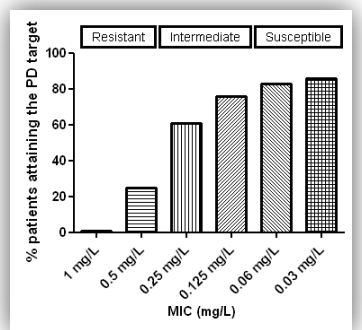

Figure 5. PD target attainment rates

CONCLUSIONS

- ✓ The in vitro activity of posaconazole was dependent on the drug exposure, the MIC and the resistance mechanism.
- ✓ Despite the same MIC values that two isolates were exhibiting, the activity of posaconazole was smaller against the isolate with the M220I mutation, compared with the isolate harboring the TR/L98H mutation in the *cyp51a* gene.
- ✓ Thus, isolates with different resistance mechanisms may exhibit different *in vitro* pharmacodynamics. These findings highlight potential differences in other time- and concentration-dependent PD parameters (e.g. PAFE, sub-MIC effects, etc).
- ✓ The AUC/MIC ratio that corresponds to 50% of maximum antifungal activity was estimated at 27.04 (17.68-41.35) close to the *f*AUC/MIC ratios of other azoles against *Candida albicans* and *A. fumigatus.*
- ✓ Based on Monte Carlo analysis, the susceptibility breakpoints of ≤0.0625, 0.125-0.25, ≥0.5 mg/l were determined for posaconazole and *A. fumigatus*.