

Impact of dosing frequency on azole's pharmacodynamics

against *Aspergillus fumigatus*

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Background. Voriconazole (VOR) and posaconazole (POS) represent first-line agents for therapy and prophylaxis of invasive aspergillosis. The emergence of azole resistance in *Aspergillus fumigatus* (AFM) has led to intense research in optimizing antifungal treatment against these isolates. Preclinical studies using animal models employed once daily regimens for these azoles when twice daily regimens are used in clinical practice. We therefore assessed the impact of dosing frequency on azoles' pharmacodynamics against AFM isolates using an *in vitro* pharmacokinetic (PK) - pharmacodynamic (PD) model.

Methods. Four clinical AFM isolates previously tested in an experimental model of aspergillosis (Mavridou et al AAC 2010 vol. 54 is. 11 and 2) were studied: one wild type isolate with no cyp51a mutation and three isolates with the cyp51a mutations G54W, M220I, and TR/L98H. The CLSI MICs were 0.125, 0.125, 0.25 and 2 mg/l for VOR and 0.03, 16, 0.5 and 1 mg/L for POS, respectively. The exposure-response relationships of VOR and POS against those isolates were determined in an *in vitro* PK-PD model where animal (q24 dosing) and human (q12 dosing) serum PK were simulated. Drug concentrations were assessed with a bioassay and the % fungal growth was estimated measuring galactomannan production for each dose and isolate. The AUC_τ/MIC vs. growth relationship was analyzed with the nonlinear regression analysis using the E_{max} model where AUC_τ is the area under the time-concentration curve for dosing interval τ of 12h for animal PK and 24h for human PK. The AUC_τ/MIC associated with 50% of maximal activity (EC50) was calculated for both drugs and dosing frequencies and differences were assessed statistically with the F test (p<0.05).

Results. The PK-PD relationship for both agents followed a sigmoid curve (R²>0.94). For VOR, the EC50 for animal q24 dosing regimens was 28.6 (16.2-50.6) AUC₀₋₂₄/MIC and it was not different than the EC50 for human q12 dosing regimens which was 24.7 (17.9-35.6) AUC₀₋₁₂/MIC. However, for POS, the EC50 for animal q24 dosing regimens was 172 (123-226) AUC₀₋₂₄/MIC and it was significantly higher than the EC50 for human q12 dosing regimens which was 27.05 (17.5-41.4) AUC₀₋₁₂/MIC.

Conclusions. The dosing frequency had a severe impact on POS but not on VOR pharmacodynamics possibly because of a time-dependent action that POS exerted.

Invasive pulmonary aspergillosis (IPA) is a life-threatening complication affecting hematological neutropenic patients and haemopoietic stem cell transplant recipients particularly those with graft-versus-host disease. Among the different *Aspergillus* species implicated in these infections, *Aspergillus fumigatus* is the most common cause of IPA.

Voriconazole (VOR) and posaconazole (POS) belong to the second generation azoles and represent the first choice antifungals for treatment and prophylaxis, in respect, against IPA. Despite the efficacy of these agents against *Aspergillus* spp. the emergence of azole resistance in *Aspergillus fumigatus* (AFM) has led to intense research in optimizing antifungal treatment against these isolates.

Beside all advances in the management of invasive aspergillosis important questions remain unresolved. Preclinical studies using animal models employed once daily regimens for these azoles when twice daily regimens are used in clinical practice.

We therefore assessed the impact of dosing frequency on azoles' pharmacodynamics against *A. fumigatus* isolates using an *in vitro* pharmacokinetic (PK) - pharmacodynamic (PD) model.

Isolates: Four clinical *A. fumigatus* isolates previously tested in an experimental model of aspergillosis (Mavridou et al AAC 2010 vol. 54 is. 11 and 2) were studied: one wild type isolate with no cyp51a mutation and three isolates with the cyp51a mutations G54W, M220I, and TR/L98H. The CLSI MICs were 0.125, 0.125, 0.25 and 2 mg/l for VOR and 0.03, 16, 0.5 and 1 mg/L for VOR, respectively. MIC differences among isolates were verified with the gradient concentration MIC test strips (Liofilchem®, Italy). Inocula were prepared in sterile saline with 1% Tween 20 in order to obtain a final concentration of 10³ CFU/mL.

In vitro PK-PD model: A previously described *in vitro* closed PK-PD diffusion/dialysis model simulating human PKs was used in this study (Meletiadis J, AAC 2012; 56: 403-10) (Figure 1). Briefly, the model consists of two departments: A) an internal (IC) corresponding to a 10 mL semi-permeable dialysis tube allowing the free diffusion of molecules with MW <20 kDa, B) an external (EC) corresponding to a 700 mL glass beaker) whose content is diluted by C) a peristaltic pump at the same rate as the clearance of the antifungal drugs in human plasma.

In vitro pharmacokinetics: The exposure-response relationships of VOR and POS against those isolates were determined in an *in vitro* PK-PD model where animal (q24 dosing) and human (q12 dosing) serum PK were simulated. More specifically, based on previously published data regarding the C_{max} of those agents in animals and in human patients, the targeted maximum concentrations (C_{max}) for POS were 9, 3 and 1 mg/L (q24 dosing) and 2.25, 0.85 and 0.15 mg/L (q12 dosing), while on the other hand the targeted C_{max} for VOR were 36.49, 11.09 and 1.60mg/L (q24 dosing) and 7, 3.5, 1.75 and 0.83mg/L (q12 dosing). The concentrations of POS and VOR were assessed with bioassay (Meletiadis J, AAC 2012; 56: 403-10).

In vitro pharmacodynamics: The percentages (%) of fungal growth at each VOR and POS dose was calculated on the basis of galactomannan production in the inoculated dialysis tubes using a commercially available sandwich enzyme-linked immunoassay (Platelia Aspergillus, Biorad). The % growth inhibition was calculated as 100%-% fungal growth.

Analysis. The AUC_τ/MIC, T>MIC and C_{max}/MIC vs. growth inhibition relationships were analyzed with the nonlinear regression analysis using the E_{max} model where AUC_τ is the area under the time-concentration curve for dosing interval τ of 12h for animal PK (q24 dosing) and 24h for human PK (q12 dosing), T>MIC is the time that concentration are higher than the MIC and C_{max}/MIC is the ratio of maximum concentration over MIC. The PK/PD parameter associated with 50% of maximal activity (P₅₀) was calculated for both drugs and dosing frequencies and differences were assessed statistically with the F test (p<0.05).

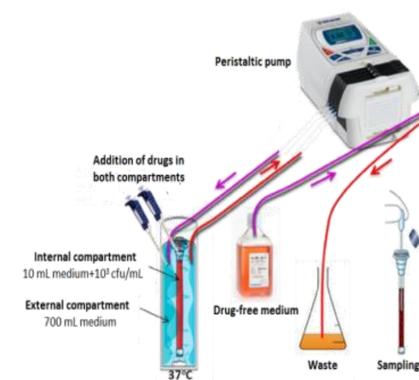


Figure1. In vitro PK/PD model.

- The PK-PD relationships for both agents correlating the % of fungal growth inhibition versus log₁₀(AUC/MIC), log₁₀(C_{max}/MIC) and log₁₀% T>MIC followed a sigmoid curve (R²>0.94) (Figure 2 and 3).
- For VOR (Figure 2), the P₅₀ for animal q24 dosing regimens was 28.9 (14.8-56.2) AUC₀₋₂₄/MIC and 5.59 (5.592-5.595) C_{max}/MIC and 52.5% (38-70%) and they were similar to P₅₀ values estimated from human q12 dosing regimens, 25.6 (16.1-40.9) AUC₀₋₁₂/MIC, 3.76 (2.47-5.58) and 53.7 (30-81%), respectively.
- However, for POS (Figure 3), the P₅₀ for animal q24 dosing regimens were 172.1 (123-226) AUC₀₋₂₄/MIC, 9.8 (6.8-14) C_{max}/MIC and 99% T>MIC and they were significantly higher than the EC50s for human q12 dosing regimens which were 22.6 (16-31.6) AUC₀₋₁₂/MIC, 2.4 (1.1-5.1) C_{max}/MIC and 81.6% T>MIC (P<0.05).

RESULTS

Figure 2. PK-PD relationships for VOR. Similar values of P₅₀ obtained by q24 and q12 dosing regimens.

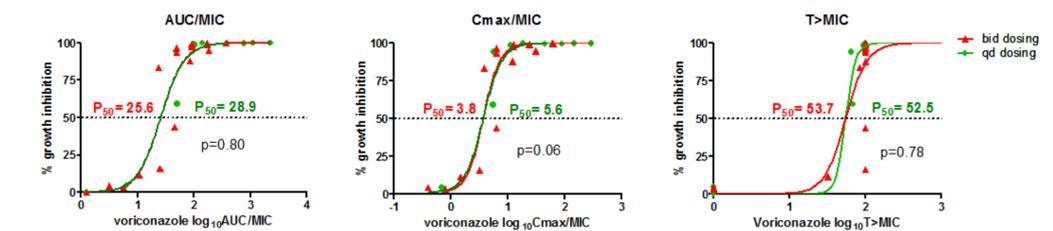
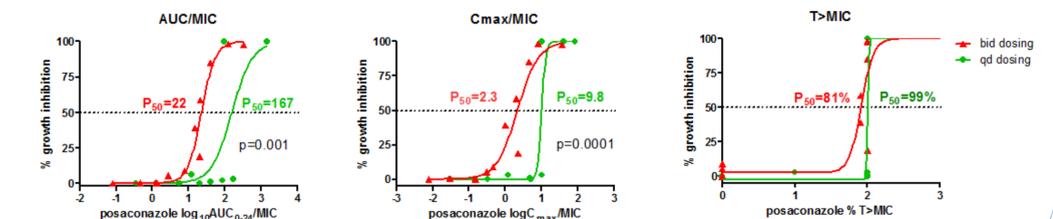


Figure 3. PK-PD relationships for POS. Significantly higher values of P₅₀ obtained by q24 compared to q12 dosing regimens.



CONCLUSIONS

- ✓ All three PK-PD parameters including T>MIC described well POS and VOR pharmacodynamics against *A. fumigatus*.
- ✓ Dosing frequency had a significant impact on POS pharmacodynamics but not on VOR pharmacodynamics. When POS was administered q24 the AUC/MIC required for the suppression of galactomannan production by 50% was significantly higher than the corresponding one when POS was administered q12.
- ✓ This effect may be due to a stronger time dependent activity that POS exerts compared to VOR. POS generally exhibits a short post-antifungal effect (PAFE) after short exposure. However longer PAFE may be detected after long exposure due to the prolonged time demanded for lanosterol depletion (Manavathu JAC 2004). More than 99% killing was previously observed at supra MIC concentrations after 48h of incubation (Manavathu JAC 2000).
- ✓ Based on these findings, the new intravenous formulation of POS should be given q12 despite that high AUCs are achieved.