Aspergillus fumigatus with priorazole exposure decreases amphotericin B sensitivity in vitro

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Introduction
Aspergillus fumigatus is a ubiquitous fungal pathogen responsible for aspergillosis in immuno-compromised individuals. Triazoles are the mainstay treatment for majority of fungal infections. Acquiredazole resistance in Aspergillus fumigatus is increasingly common and is a significant cause of treatment failure. This may result in switching the class of antifungal drug, though the patient may not improve clinically.

Aims
The aim of this study was to investigate the impact of prior azole exposure on subsequent amphotericin B (AMB) sensitivity in A. fumigatus biofilms. Based on our previous studies on adaptive resistance mechanisms in fungal biofilms, we hypothesised that azole selection pressure activates heat shock protein 90 (Hsp90) and increases extracellular DNA (eDNA) release in A. fumigatus biofilms, therefore affecting AMB sensitivity.

Materials and Methods

- For time-kill kinetics AMB (1 mg/L) & voriconazole (VRC [1 mg/L]) either alone or in combination (VRC-AMB) were prepared in RPMI and tested against germlings (8 h)

Susceptibility testing
- The MIC50 of AMB ± DNase (128 mg/L) or geldanamycin (GDA [50 mg/L]) was determined by standard CLSI broth microdilution method
- For azole pre-treatment germlings were treated with VRC at a sub MIC concentration of 0.06 mg/L for 24 h
- Cellular viability determined by the XTT assay

Imaging
For scanning electron microscope (SEM), biofilms grown and treated on Thermaxx coverslips then biofilms were fixed by aldehydes and viewed under a JEOL JSM-6400 SEM.

Results & Discussion
Pharmacodynamics of VRC, AMB alone and in combination against A. fumigatus germlings

Effect of AMB ± DNase/GDA against azole treated A. fumigatus

Figure (i) shows the time-kill kinetics of AMB, VRC or VRC-AMB against germinated conidia (8 h). * p<0.001.

Figure (ii) shows the MIC50 of AMB ± DNase or GDA against either VRC pre-treated (VRC PT) or untreated (-) A. fumigatus.

Figure (iii) SEM images shows the effect of AMB (1 mg/L) against VRC -PT A. fumigatus biofilm.

Figure (iv) AMB absorbance spectra showing the binding of AMB (8 mg/L) with A. fumigatus biofilms (24 h) over the period of 2 h. Bar graph shows the binding of AMB with biofilms in the presence and absence of extracellular matrix (ECM). * p<0.05

Summary
These data show that A. fumigatus exposure to azole drug induces eDNA release and activates the stress response, which collectively confers AMB resistance in vitro. Pharmacological inhibition of these mechanisms may provide novel therapeutic strategies following ineffectual azole therapy.