Evaluation of antifungal drugs by an invasive aspergillosis-on-chip model

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Introduction:

Invasive fungal infections by the mould *Aspergillus fumigatus* in immunocompromised patients are significantly increasing. Infection occurs in the lung where fungal conidia germinate to grow into filamentous bodies (hyphae), penetrate the epithelium and invade into the bloodstream. Invasive aspergillosis is associated with high mortality rates and limited treatment options. Testing of new antifungal drugs *in vitro* is insufficient, making animal models the gold-standard for drug efficacy testing. To overcome *in vitro* limitations, organ-on-chip systems mimic human physiology much more closely, thus providing a more suitable drug testing environment in terms of organ structure, cell types and dynamics (e.g. flow). Here, we used our recently established "invasive aspergillosis-on-chip" (IAC) model to re-capitulate the treatment of *A. fumigatus* infection by the well-established drugs Voriconazole (VOR), Caspofungin (CAS) and the new antifungal agent Jagaricin (JAG).

Jagaricin (JAG)

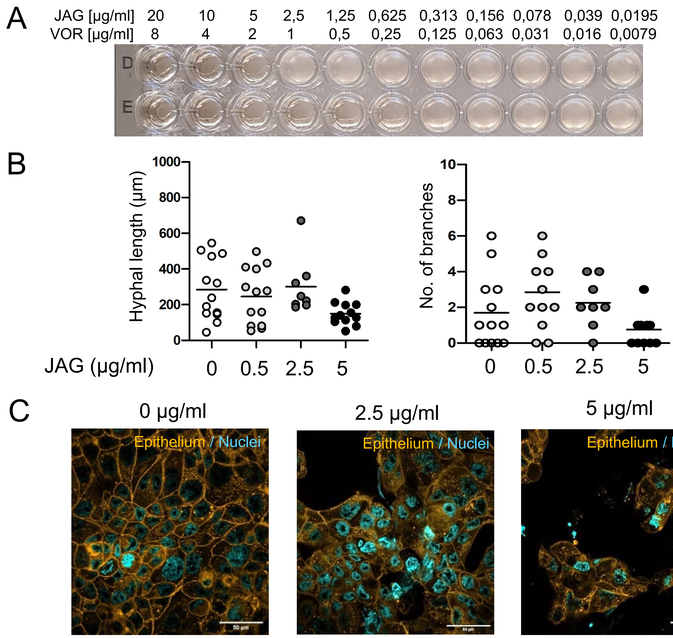

Figure 3: The novel agent Jagaricin inhibited *A. fumigatus* growth but compromised the epithelium at antifungal concentrations when grown in the IAC model with JAG perfusion at indicated concentrations. A) Microdilution of JAG and VOR on *A. fumigatus* B) Characteristics of *A. fumigatus* hyphae from n=3 independent experiments (One-way ANOVA with p >0.05). C) Alveolar epithelium in absence (0 µg/ml) and presence of JAG stained by E-cadherin (orange) and nuclei by Hoechst 33258. Scale bars: 50 µm

Invasive aspergillosis-on-chip model

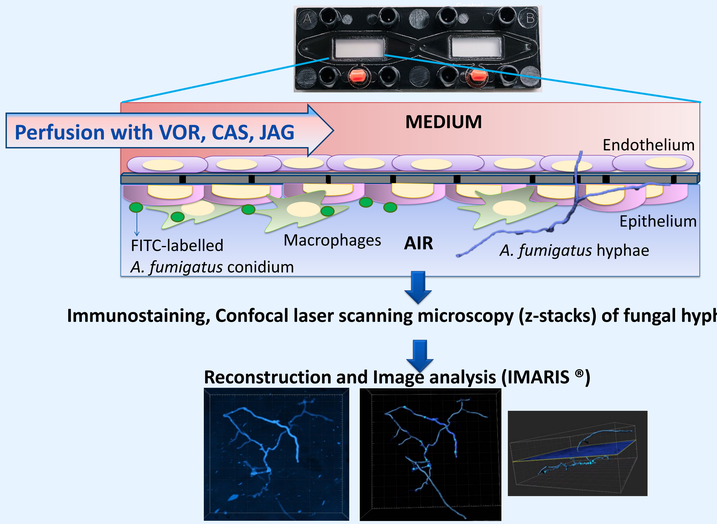

Figure 1: Setup and analysis of the invasive aspergillosis-on-chip (IAC) model which included a porous membrane, an upper (blood) chamber comprising human endothelial cells and perfused with medium (and antifunal drugs), and a lower (alveolar) chamber including human epithelial cells and monocyte-derived macrophages at an air-liquid interface. Labelled *A. fumigatus* conidia were applied to the lung side and incubated over-night to give rise to hyphae. Confocal laser scanning microscopy was employed to obtain z-stack images of hyphae, which were quantified by advanced automated image analysis (IMARIS® software).

Voriconazole (VOR)

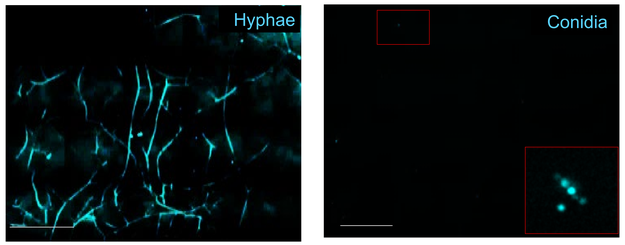

Figure 2: Prevention of hyphae formation by VOR (4 µg/ml) perfusion during over night incubation. Only ungerminated conidia were found (red insert). Scale bars: 200 µm

References: (1) Rennert et al., Biomaterials, 2015; (2) Deinhardt-Emmer et al., Biofabrication, 2019. (3) Graupner K. et al., Angewandte Chemie Int. Ed., 2012

Conclusion and outlook:

The clinically applied antifungal agents VOR and CAS performed in the IAC models as expected. However, the presence of invasive hyphae in CAS-treated samples, not detected in previous tests that only look at horizontal growth, highlight the necessity of *in vivo*-like test systems. In long run, organ-on-chip models may provide a much-needed tool to overcome animal testing.

Caspofungin (CAS)

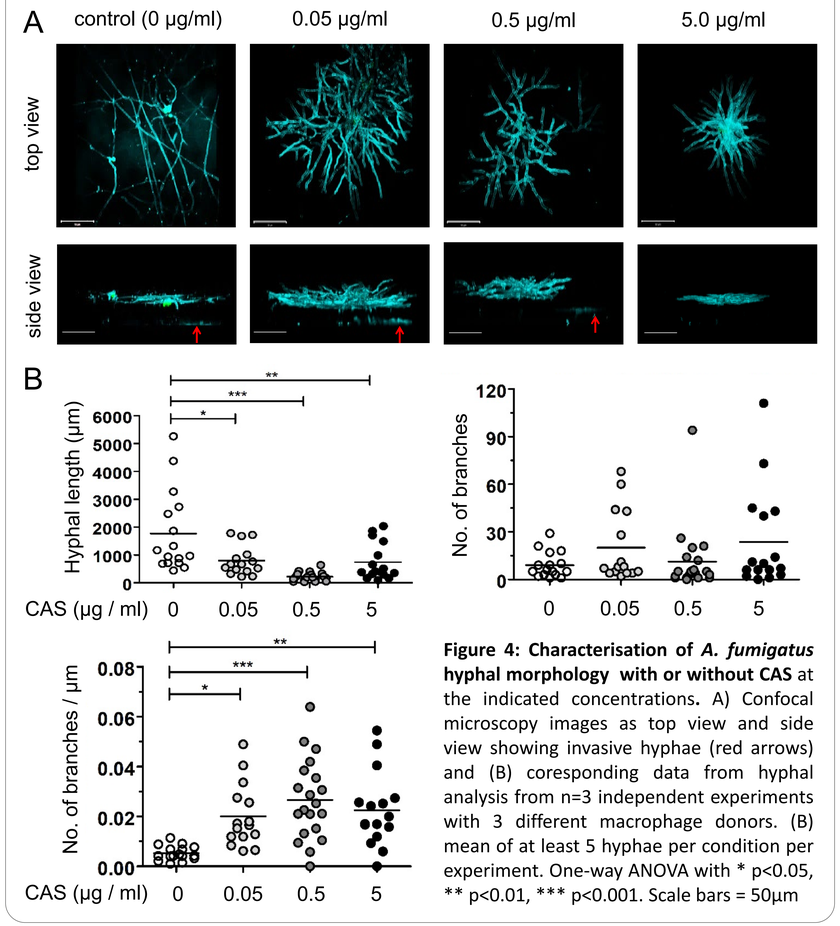

Figure 4: Characterisation of *A. fumigatus* hyphal morphology with or without CAS at the indicated concentrations. A) Confocal microscopy images as top view and side view showing invasive hyphae (red arrows) and (B) coresponding data from hyphal analysis from n=3 independent experiments with 3 different macrophage donors. (B) mean of at least 5 hyphae per condition per experiment. One-way ANOVA with * p<0.05, ** p<0.01, *** p<0.001. Scale bars = 50µm