Synergic anti-fungal activity of topical PC945, a novel inhaled azole, with systemic echinocandin on *Aspergillus fumigatus in vitro* human alveoli model

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Introduction/Aim

PC945 is a novel antifungal agent[1,2] being developed as an inhalation therapy for the treatment of aspergillosis (Phase 2). We previously reported that topical PC945 and systemic azoles showed synergic antifungal effects *in vitro* and *in vivo*[3]. This study examined the anti-fungal activity of PC945 on growth of *Aspergillus fumigatus* (*A. fumigatus*) as a combination therapy with echinocandins *in vitro* using a model of human alveoli.

Methods

A model of human alveoli was constructed in transwells consisting of a bilayer of human alveolar epithelial and endothelial cells. PC945 (0.1 µg/mL) was either added as monotherapy once daily on days 0, 1 and 2 to the epithelial compartment (upper chamber) mimicking inhalation treatment or was combined with caspofungin or micafungin (1 µg/mL) added to the endothelial compartment (lower chamber) mimicking oral treatment. Transwells were infected with azole sensitive (NCPF2010) *A. fumigatus* at a concentration of 1 x 10^4 spores/mL, one hour after treatment on day 0 (Fig.1). Subsequently, levels of galactomannan in the bottom chamber, which was collected Day 1 to Day 5 post inoculation, were quantified by Platelia™(Biorad). Azole-resistant *A. fumigatus* harbouring TR34/L98H mutation was also infected in the presence of PC945 (1 µg/mL, upper) with/without caspofungin (0.1 µg/mL, lower). Figure 2 shows histology of non-infected and *A.fumigatus* infected controls (Day3).

Figure 1. Treatment schedule

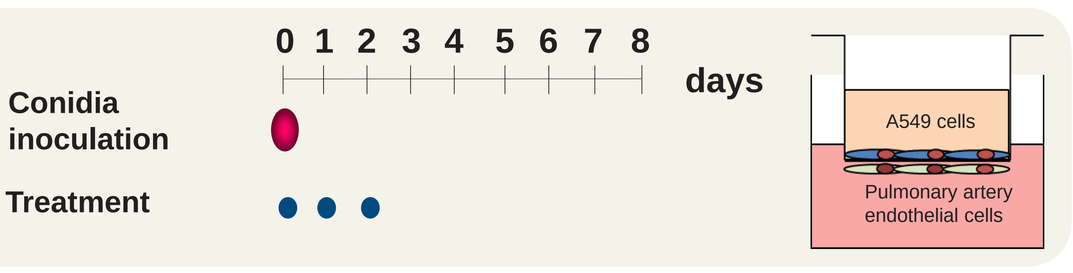
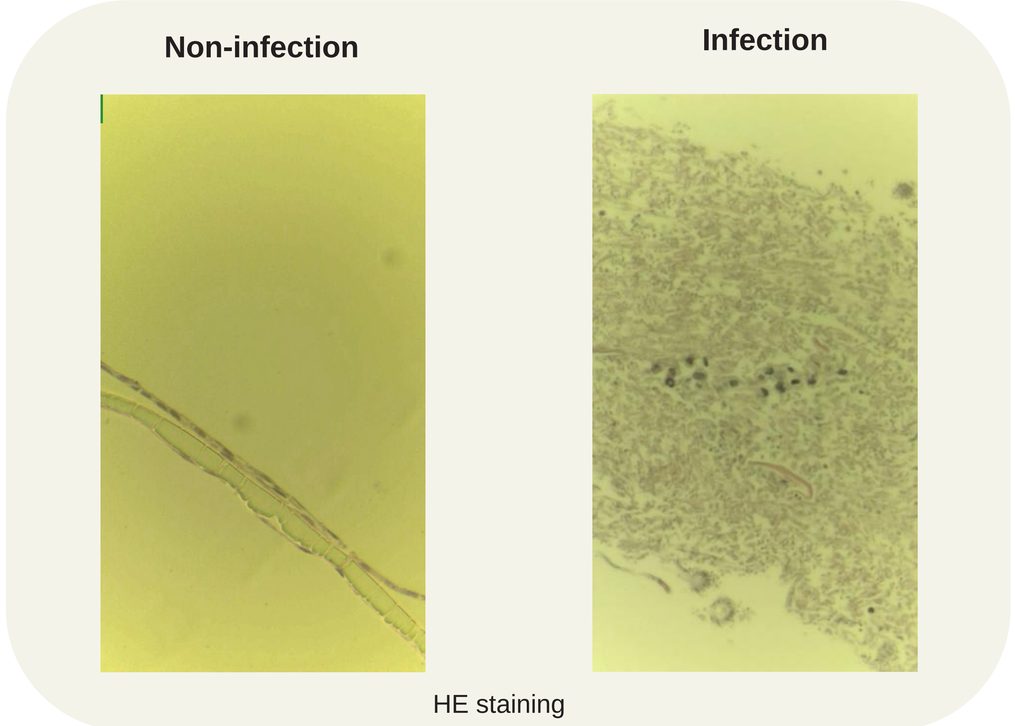


Figure 2. Representative image of non-infected and *A.fumigatus* infected controls (Day 3)



Results

Exp 1: Azole susceptible strain (NCPF2010) infection

In the *in vitro* model of human alveoli, caspofungin or micafungin (1 µg/mL; lower chamber) or PC945 (0.1 µg/mL; upper chamber) as monotherapy had only mild inhibitory effects on azole susceptible *A. fumigatus* invasion Day 1 post infection, whereas combined treatment with PC945 and caspofungin/micafungin achieved marked inhibition of fungal invasion up to Day 5 post infection (Fig.3).

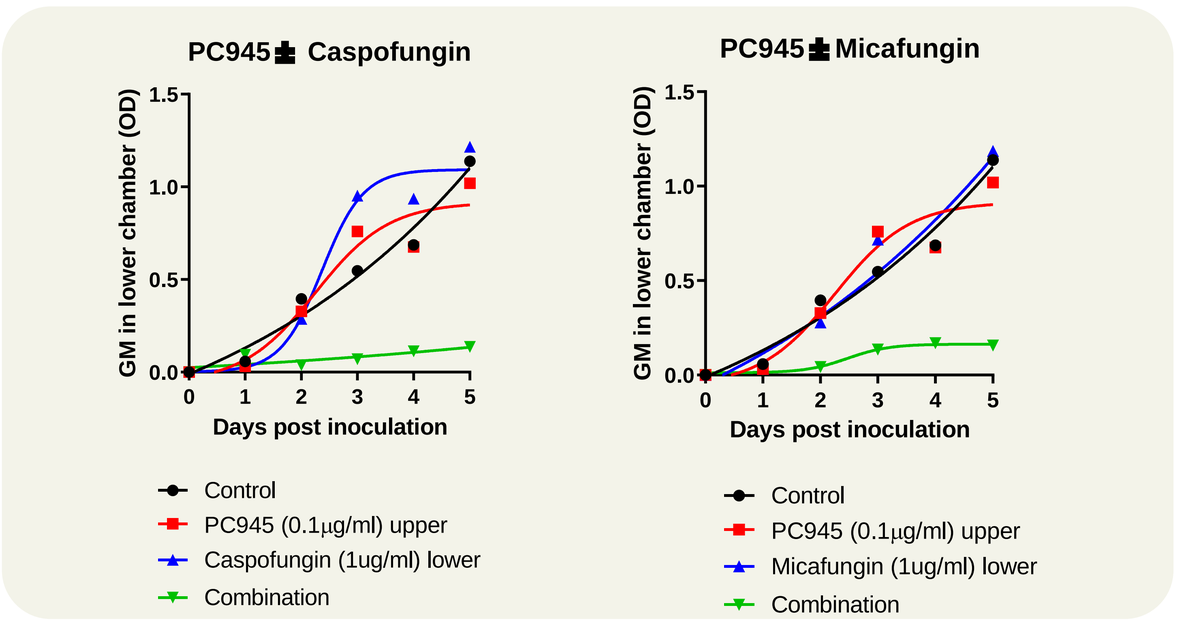


Figure 3. Time-dependent changes in GM concentrations in lower chamber after azole-susceptible *A. fumigatus* infection to the upper chamber in the presence or absence of echinocandin (lower chamber), PC945 (upper chamber) or combination. Compounds were treated daily (Day 0 to Day 2), and samples were collected daily for analysis.

Exp 2: Azole resistant strain (TR34 L98H) infection

A similar pattern was observed for the azole resistant strain TR34-L98H, with monotherapy (caspofungin,1 µg/mL, lower; PC945, 1 µg/mL, lower) monotherapy weakly inhibiting fungus invasion. In contrast, combined treatment with PC945 and caspofungin provided much greater protection than monotherapy (Fig. 4).

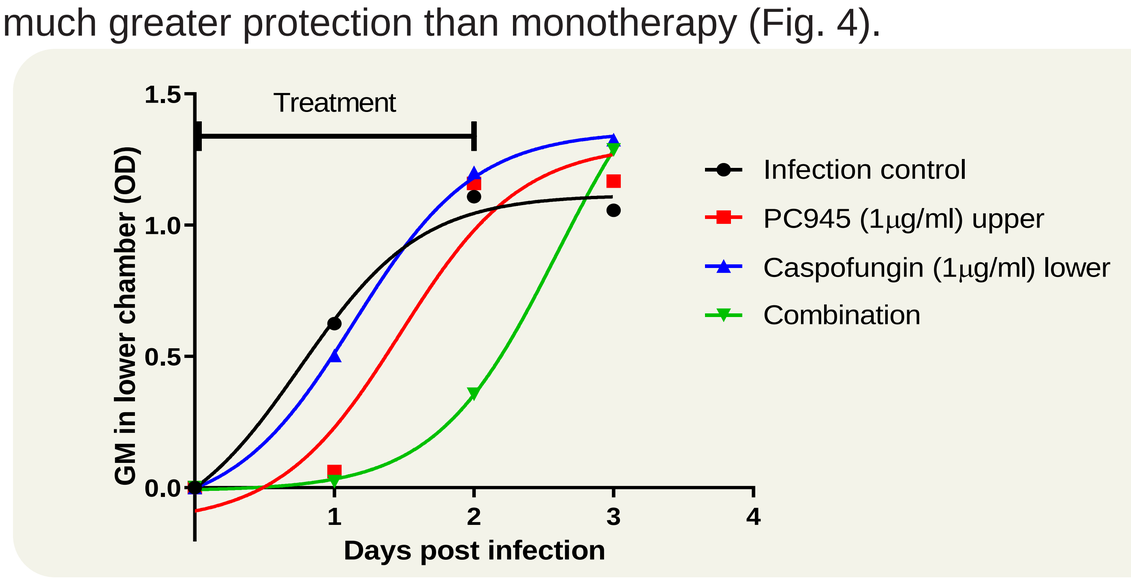


Figure 4. Time-dependent change of GM in lower chamber after azole-resistant *A. fumigatus* infection to the upper chamber in the presence or absence of caspofungin (lower chamber), PC945 (upper chamber) or combination. Compounds were treated daily on Day 0 to Day 2, and samples were collected daily.

Conclusion

In this study, combined treatment with PC945 and echinocandins was shown to inhibit azole-susceptible and azole-resistant *A. fumigatus* growth to a greater extent than monotherapy using either compound. PC945 therefore has the potential to be used in combination with established antifungal drugs for the treatment of azole sensitive and resistant *A. fumigatus* infection in humans.

References

1. Colley T et al., Antimicrob Agents Chemother. 2017 Apr 24;61(5). pii: e02280-16.
2. Kimura G et al., Antimicrob Agents Chemother. 2017 Aug 24;61(9). pii: e00124-17.
3. Colley T et al., Sci Rep. 2019 Jul 1;9(1):9482.