Altered *A.fumigatus* cell wall integrity after exposure to PC945, a novel inhaled azole

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Introduction/Aim

PC945 is a novel antifungal agent being developed as an inhalation therapy for the treatment of aspergillosis[1,2]. We previously found that PC945 treatment was more effective in *in vivo A. fumigatus* infected mice than posaconazole although both compounds showed similar anti-fungal effects in MIC assay by broth microdilution *in vitro*[1.2]. Therefore, our hypothesis is that PC945 alters *A. fumigatus* cell wall integrity, leading to antigen being exposed to immune cells and consequently more efficient fungal clearance.

Methods

Experiment 1: Congo red (CR) and calcofluor white (CFW) are cell wall perturbing agents that influence cell wall chitin and β-glucan deposition, respectively. *A. fumigatus* conidia (strain AF293)[10 – 1x10^5 conidia] was spotted onto Saboraud agar containing CR (10 mg/ml) or CFW (10 mg/ml) with/without DMSO (vehicle) or PC945 (0.008 µg/ml), and the plates were incubated at 37°C, 5% CO_2 for 24 h.

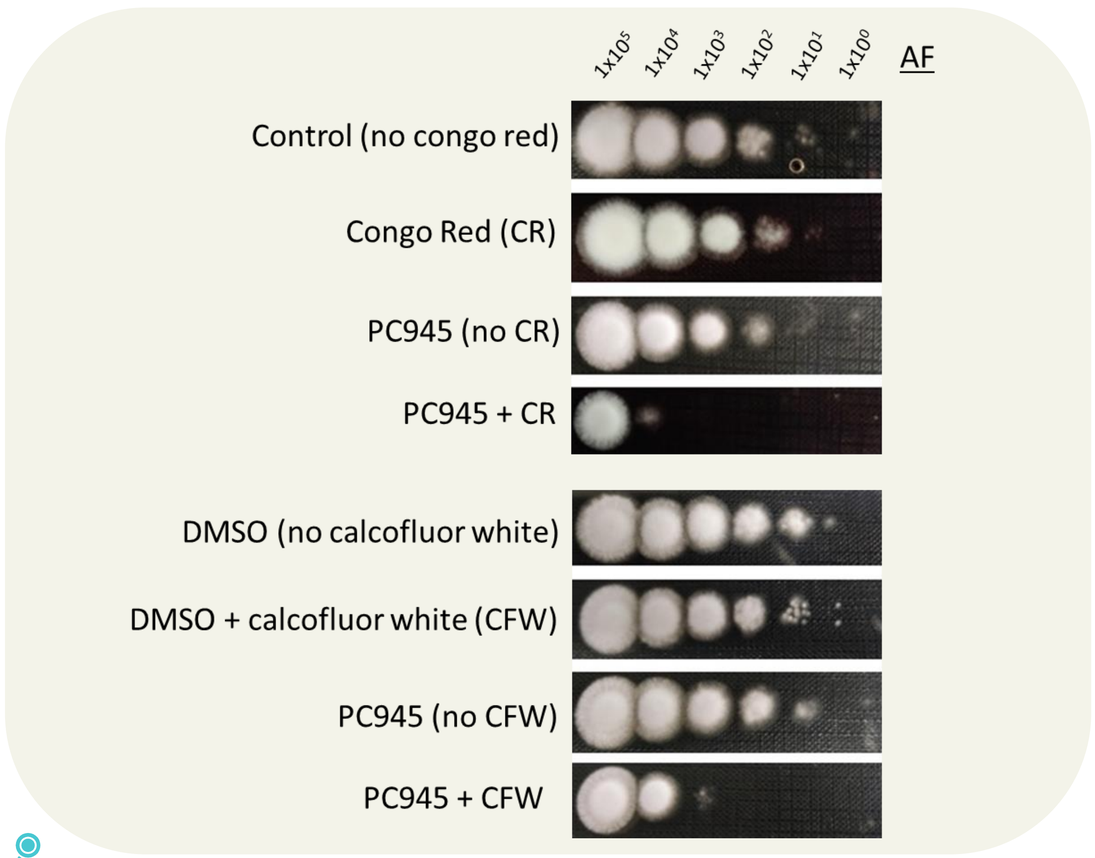
Experiment 2: to examine the presentation of β-glucan and chitin expression on the cell wall of *A. fumigatus* (strain AF293), the fungus was stained with fc-dectin-1a (human dectin-1a fused to human IgG1 fc domain) (1µg/ml; Invivogen) with AlexaFluor-647 conjugated anti-human antibody and FITC-conjugated wheat germ agglutinin (WGA-FITC; 1µg/ml; Sigma) after 3hour incubation with PC945. The level of fluorescent was detected by flow cytometer.

Experiment 3: A*. fumigatus* (strain AF293) was treated with compound or vehicle (0.5% DMSO) and incubated for 24 h at 37°C, 5% CO_2. *A. fumigatus* was inactivated by UV exposure using a Stratalinker UV Crosslinker 1800 (dose, 4 × 105 mJ/cm2). PBMCs obtained from healthy volunteers were exposed to UV-inactivated PC945 loaded *A. fumigatus* for 24 h at 37°C, 5% CO_2, and IL-1β in the supernatant was measured by ELISA (R&D systems) .

Results

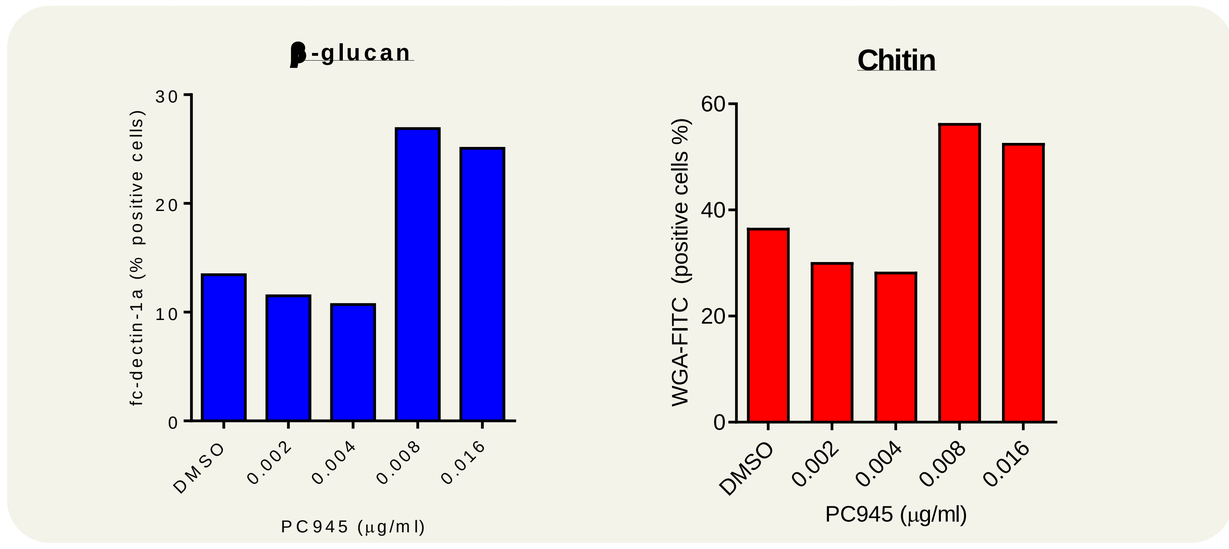
Experiment 1

PC945 enhanced susceptibility of *A. fumigatus to* CR and CFW, suggesting that PC945 (0.008µg/mL) compromised cell wall integrity. Posaconazole also showed similar effects at 2 fold higher concentration (0.016µg/mL).



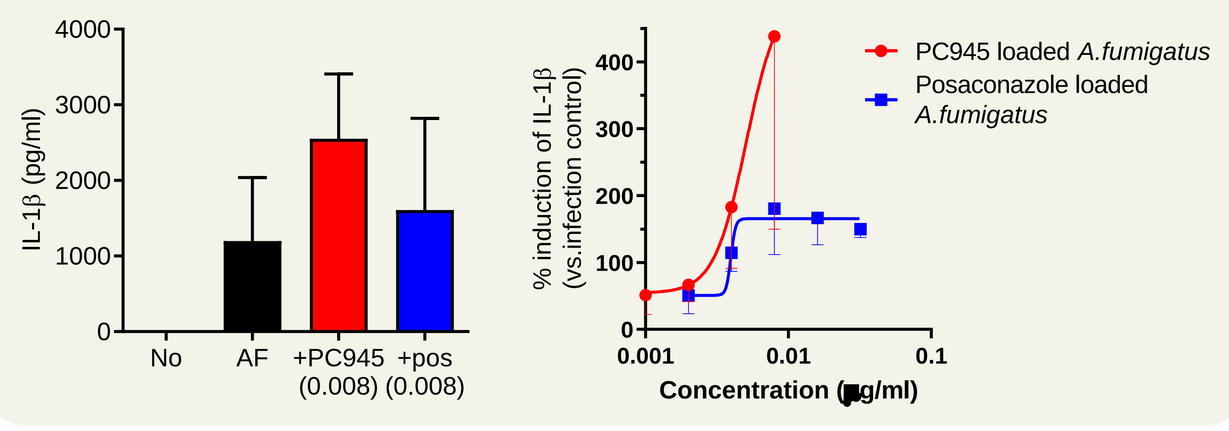
Experiment 2:

Treatment of *A. fumigatus* with PC945 resulted in greater detection of β-glucan and chitin on the fungal surface, detected by fc-dectin-1a and WGA-FITC, respectively. This data suggests that either expression of β-glucan and chitin on *A. fumigatus* is altered in response to PC945, or detection of these epitopes is increased potentially due to compromised cell wall integrity.

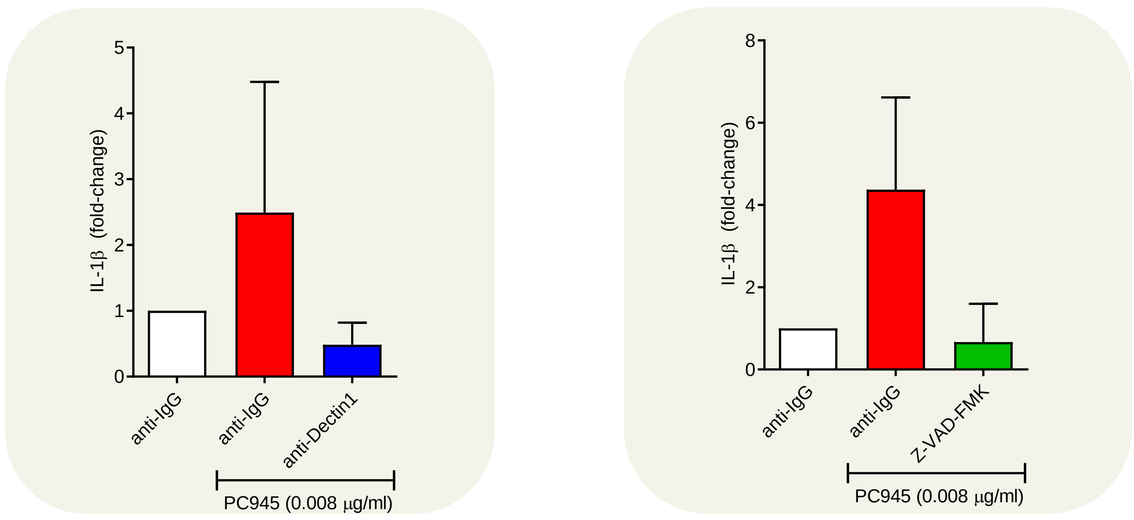


Experiment 3:

UV-inactivated PC945 loaded *A. fumigatus* induced IL-1β production from human PBMCs in a PC945 concentration-dependent manner. However, posaconazole loaded *A.fumigatrus* showed limited IL-1β production.



The induction of IL-1β by PC945 (0.008 µg/ml) treated *A. fumigatus* was attenuated by the presence of neutralising antibodies against Dectin-1 (left Fig below). Thus, the enhanced inflammatory response generated against PC945 treated *A. fumigatus* is dependent on Dectin-1 mediated signalling pathways via activation of the inflammasome. The induction of IL-1β by PC945 treated *A. fumigatus* was also attenuated by the presence of the pan-caspase inhibitor Z-VAD-FMK (right Fig below). Therefore, the enhanced inflammatory response generated against PC945 treated *A. fumigatus* is dependent on caspase-mediated signalling pathways and suggests possible enhanced activation of the inflammasome by PC945-treated *A. fumigatus*.



Conclusion

This data suggests that β-glucan and chitin on *A. fumigatus* are exposed after PC945 treatment due to compromised cell wall integrity, and it is possible that PC945 induces increased recognition of *A. fumigatus* to immune cells to accelerate clearance of fungal body.

Reference

1. Colley T et al., *In Vitro* and *In Vivo* Antifungal Profile of a Novel and Long-Acting Inhaled Azole, **PC945**, on Aspergillus fumigatus Infection. Antimicrob Agents Chemother. 2017 Apr 24;61(5). pii: e02280-16.
2. Kimura G et al., *In Vivo* Biomarker Analysis of the Effects of Intranasally Dosed **PC945**, a Novel Antifungal Triazole, on Aspergillus fumigatus Infection in Immunocompromised Mice. Antimicrob Agents Chemother. 2017 Aug 24;61(9). pii: e00124-17.

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