Introduction/Aim

PC945 is a novel antifungal agent being developed as an inhalation therapy for the treatment of aspergillosis. We previously found that PC945 treatment was more effective in vivo A. fumigatus infected mice than posaconazole although both compounds showed similar anti-fungal effects in MIC assay by broth microdilution in vitro. 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) (10–1x10⁶ 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% CO₂ 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 IgG1c domain) (1μg/ml; Invivogen) with AlexaFluor-647 conjugated anti-human antibody and FITC-conjugated wheat germ agglutinin (WGA-FITC; 1μg/ml; Sigma) after 3 hour incubation with PC945. The level of fluorescent was detected by flow cytometry.

Experiment 3: A. fumigatus (strain AF293) was treated with compound or vehicle (0.5% DMSO) and incubated for 24 h at 37°C, 5% CO₂. 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% CO₂, 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

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