Introduction
PC945 is a novel antifungal triazole developed as an inhalation therapy for the treatment of lung aspergillosis. In this study, we examined the effects of intranasally dosed PC945 on fungus and cytokine biomarkers in Aspergillus fumigatus infected, immunocompromised mice.

Methods

- A/J mice (males, 5 weeks old) were dosed with hydrocortisone (125 mg/kg, sc) on days 3, 2, and 1 before infection, and with cyclophosphamide (250 mg/kg, ip) 2 days before infection to induce temporary neutropenia.
- On day 0, animals were infected intranasally with 30 μL of the spore suspension of Aspergillus fumigatus (ATCC 13073) at a concentration of 1.67 x 10^8 spores mL^-1 of physiological saline.
- PC945 was treated intranasally on days 1, 2, and 3 post infection and animals were culled 24 hours after the final treatment on day 3 (day 4).
- Bronchoalveolar lavage fluid (BALF) and serum were collected for biomarker analysis. Bronchial epithelial lining fluid (ELF) was also collected using a synthetic absorptive matrix (Nasosorption™ SAM strips: http://www.huntdevelopments.co.uk).
- Biomarkers were assessed in mice alive at day 4.

A/J male mice
Cyclophosphamide (250mg/kg, ip)
Hydrocortisone (125mg/kg, sc)

Drinking water (tetacycline 1mgL + ciprofloxacin 64mgL)

-3 -2 -1 0 1 2 3 4 days

A.f infection (in)
Treatment (in)
Cull, Sampling

Results

CFU in Lung
The high level of fungal load (CFU) was detected in Aspergillus infected mice lung. PC945 inhibited CFU in Aspergillus infected mice lung in a dose-dependent manner (Fig.1). It also inhibited AF DNA content in lung, evaluated by PCR (data not shown).

Galactomannan (GM) in BALF and serum

The high level of GM was detected in BALF and serum from Aspergillus infected mice. PC945 inhibited GM in Aspergillus infected mice in a dose-dependent manner (Fig.2, 3).

Galactomannan (GM) in ELF on trachea and BALF collected by SAM strip

ELF was collected by attaching a SAM strip (3mm x 7mm) in inner wall of trachea before BAL or spiking SAM in fresh BALF. The high level of GM was detected on SAM strip from trachea or BALF from Aspergillus infected mice. PC945 inhibited GM in a dose-dependent manner (Fig.4, 5).

Biomarkers in BALF and serum

PC945 dose-dependently inhibited malondialdehyde (MDA), IL-17 and CXCL1 in BALF and TNF-α and IL-6 in serum, which were increased by Aspergillus infection (Fig. 6-10).

ID_{50} of PC945 (mg/kg, intranasal)

<table>
<thead>
<tr>
<th>Macrophage BALF</th>
<th>CFU Lung</th>
<th>GM BALF</th>
<th>GM serum</th>
<th>GM SAM-ELF</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.78</td>
<td>0.067</td>
<td>0.015</td>
<td>0.012</td>
<td>0.021</td>
</tr>
<tr>
<td>MDA BALF</td>
<td>IL-17 BALF</td>
<td>CXCL1 BALF</td>
<td>IL-6 serum</td>
<td>TNF-α serum</td>
</tr>
<tr>
<td>0.079</td>
<td>0.20</td>
<td>0.014</td>
<td>0.091</td>
<td>0.12</td>
</tr>
</tbody>
</table>

*ID_{50} values were calculated assuming 25g of body weight and 60% efficiency of intranasal treatment.

Conclusion

- Therapeutic intervention with intranasally dosed PC945 exhibited potent inhibitory effects of fungal load (culture and PCR), GM concentrations and A. fumigatus-dependent inflammation.
- Thus, PC945 has the potential to be a novel inhaled therapy for the treatment of A. fumigatus infection in humans.
- In addition, we were able to detect fungal biomarkers in ELF in bronchus collected by sampling with Nasosorption™ SAM strips, and this sampling method will be potentially less invasive and useful for biomarker analysis of respiratory fungal infection in humans.