Safety and nonclinical and clinical pharmacokinetics of PC945, a novel inhaled triazole antifungal agent

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Abstract

Aims PC945 is a novel antifungal triazole for nebulised delivery to treat lung *Aspergillus* infections. Pharmacokinetic and safety profiles from nonclinical studies and clinical trials in healthy subjects and subjects with mild asthma were characterised. Methods Toxicokinetics were assessed following daily 2-hour inhalation for 14 days. Drug-drug interactions were evaluated using pooled human liver microsomes. Clinical safety and pharmacokinetics were assessed following (i) single inhaled doses (0.5–10 mg), (ii) 7-day repeat doses (5 mg daily) in healthy subjects; (iii) a single dose (5 mg) in subjects with mild asthma. Results C_{max} occurred 4 hours (rats) or immediately (dogs) after a single dose. PC945 lung concentrations were substantially higher (>2000-fold) than those in plasma. PC945 only inhibited CYP3A4/5 substrate metabolism (IC_{50}: 1.33 μM [testosterone] and 0.085 μM [midazolam]). Geometric mean C_{max} was 322 pg/mL (healthy subjects) and 335 pg/mL (subjects with mild asthma) 4–5 hours (median t_{max}) after a single inhalation (5 mg). Following repeat, once daily inhalation (5 mg), Day 7 C_{max} was 951 pg/mL (0.0016 μM) 45 minutes after dosing. Increases in C_{max} and $AUC_{0–24h}$ were approximately dose proportional (0.5-10 mg). PC945 administration was well tolerated in both healthy subjects and subjects with mild asthma. Treatment-emergent adverse events were mild/moderate and resolved before the study ended. No clinically significant lung function changes were observed. Conclusions PC945 pharmacokinetics translated from nonclinical species to humans showed slow absorption from lungs and low systemic exposure, thereby limiting the potential for adverse side effects and drug interactions commonly seen with systemically delivered azoles.

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Running title: PC945 safety and pharmacokinetic profiles

Keywords: PC945, inhaled administration, safety, pharmacokinetics, drug-drug interaction, first-in-human, antifungal

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- The high plasma drug concentrations required to treat pulmonary fungal infections with current systemic therapies are commonly associated with side effects and drug-drug interactions.
- PC945, a novel triazole, inhibits planktonic growth and bronchial epithelial cell infection by *Aspergillus* species *in vitro* .

WHAT THIS STUDY ADDS

- Findings from clinical and nonclinical studies demonstrated that repeat daily doses of inhaled PC945 led to prolonged absorption from the lung and minimal systemic exposure.
- PC945 was well tolerated in healthy subjects and subjects with mild asthma.
- Inhaled PC945 should have a wider therapeutic index than systemic antifungal agents.

ABSTRACT

Aims

PC945 is a novel antifungal triazole for nebulised delivery to treat lung *Aspergillus* infections. Pharmacokinetic and safety profiles from nonclinical studies and clinical trials in healthy subjects and subjects with mild asthma were characterised.

Methods

Toxicokinetics were assessed following daily 2-hour inhalation for 14 days. Drug-drug interactions were evaluated using pooled human liver microsomes. Clinical safety and pharmacokinetics were assessed following (i) single inhaled doses (0.5-10 mg), (ii) 7-day repeat doses (5 mg daily) in healthy subjects; (iii) a single dose (5 mg) in subjects with mild asthma.

Results

C_{max} occurred 4 hours (rats) or immediately (dogs) after a single dose. PC945 lung concentrations were substantially higher (>2000-fold) than those in plasma. PC945 only inhibited CYP3A4/5 substrate metabolism (IC_{50}: 1.33 μM [testosterone] and 0.085 μM [midazolam]). Geometric mean C_{max} was 322 pg/mL (healthy subjects) and 335 pg/mL (subjects with mild asthma) 4–5 hours (median t_{max}) after a single inhalation (5 mg). Following repeat, once daily inhalation (5 mg), Day 7 C_{max} was 951 pg/mL (0.0016 μM) 45 minutes after dosing. Increases in C_{max} and $AUC_{0–24h}$ were approximately dose-proportional (0.5-10 mg). PC945 administration was well tolerated in both healthy subjects and subjects with mild asthma. Treatment-emergent adverse events were mild/moderate and resolved before the study ended. No clinically significant lung function changes were observed.

Conclusions

PC945 pharmacokinetics translated from nonclinical species to humans showed slow absorption from lungs and low systemic exposure, thereby limiting the potential for adverse side effects and drug interactions commonly seen with systemically delivered azoles.

1. INTRODUCTION

Incidence of fungal infections has increased substantially over the past two decades.[1] Immunocompromised or immunosuppressed patients are particularly susceptible to such infections and invasive forms remain a leading cause of morbidity and mortality for these patients.[2] Pulmonary aspergillosis, caused by *Aspergillus* , is particularly problematic.[3] *Aspergillus fumigatus* is one of the primary causative agents of lung infections in humans.[4] Chronic *Aspergillus*infections can leave patients with permanent lung damage, requiring life-long management using oral azole treatment.[5]

Existing treatments for fungal infections are administered orally (azoles) or intravenously (azoles, amphotericin B or the echinocandins).[6] Azole antifungals are potent inhibitors of cytochrome P450 (CYP450) enzymes, especially the CYP3A4 isoenzyme, which is responsible for the metabolism of a broad range of drugs.[7] This inhibition presents a significant risk of interactions with other co-medicated drugs. To allow orally or intravenously administered antifungal agents to achieve high local concentrations sufficient for pathogen clearance, systemic exposure must be high, resulting in poor safety profiles.[8,9]

Nebulised delivery of antifungal agents results in higher local exposure in the epithelial lining fluid compared with intravenous administration.[10] PC945 is the first antifungal triazole specifically designed to treat pulmonary infection via inhaled administration.[11] In common with other triazole agents, PC945 inhibits the enzyme lanosterol 14α-demethylase (CYP51A1) in fungus, which prevents conversion of lanosterol to ergosterol.[12,13] Reduction of ergosterol causes disruption to the structure and function of fungal membranes, hence inhibiting fungal growth and spread. PC945 inhibits *in vitro*planktonic growth and bronchial epithelial cell infection by*A. fumigatus* .[13,14] In animal models, PC945 delivered a sustained and persistent antifungal effect in the lung when administered intranasally.[15] In addition, PC945 was more effective than alternative antifungal agents because of a higher local exposure at the infected site, which was attributed to the intranasal administration delivering the compound directly to the lung.[13,15]

In this article, we report the pharmacokinetic profile of PC945 in nonclinical studies after single and repeat inhaled doses in rats and dogs. We also present the results from a Phase 1 study, which evaluated the safety, tolerability and pharmacokinetics of single (escalating) and repeat inhaled doses of PC945 in healthy subjects, as well as the safety and tolerability of a single dose of inhaled PC945 in subjects with mild asthma. (ClinicalTrials.gov Identifier: NCT02715570)

2. METHODS

2.1. Drug

For nonclinical studies, PC945 was synthesised by Sygnature Discovery Ltd (Nottingham, UK). PC945 powder was directly suspended in sodium phosphate-buffered saline containing wetting agents to 10 mg/mL and further diluted with physiological saline after sonication to obtain the desired dose concentration.

For the clinical study, PC945 was manufactured by Onyx Scientific Ltd (Sunderland, UK) and supplied as a powder at a single strength of 14 mg/vial (Juniper Pharma, Nottingham, UK) for reconstitution using placebo solution. Placebo was supplied in a similar vial (Nova Laboratories, Leicester, UK). PC945 and placebo were administered by oral inhalation using a PARI LC SPRINT® nebuliser and PARI TurboBoy SX® compressor (PARI Medical Ltd., Surrey, UK).

2.2. Nonclinical toxicokinetic studies

All animal studies were designed to meet the requirements of European Parliament and Council Directive 2001/83/EC and its amendment, Commission Directive 2003/63/EC.[16,17]

PC945 was administered to rats (Han Wistar) and dogs (Beagle) at nominal doses of approximately 2, 7 and 18 mg/kg. Each dose was inhaled over a 2-hour period once daily for 14 days. Blood samples (0.3 mL for rats and 0.5 mL for dogs) were collected at six timepoints ranging from 0 to 22 hours post-end of inhalation on Day 1 (single dose systemic exposure) and on Day 14 (repeat dose exposure). Lung samples were taken from rats on Day 15 to measure PC945 lung concentrations.

2.3. *In vitro* drug-drug interactions

PC945 and posaconazole (Sigma-Aldrich, UK) were added to the pooled human liver microsomal samples (Pharmaron Rushden, UK) at concentrations of 0, 0.03, 0.1, 0.3, 1, 3 and 5 μM. The mixtures were pre-incubated at 37°C for 0 or 30 minutes before the addition of selective CYP substrates. Microsomal reactions were terminated by adding 100 μL of cold (4°C) methanol containing appropriate internal standard. Aliquots (150 μL) of each sample were centrifuged and the supernatant was used for substrate analysis by ultra-performance liquid chromatography with tandem mass spectrometry (UPLC-MS/MS) on the day of incubations.

Change in substrate concentration was measured to determine the extent of CYP-isoform inhibition. The 30-minute pre-incubations were performed to determine whether there was time-dependent inhibition of any CYP isoform (CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6 and 3A4/5). Untreated control contained only solvent, dimethyl sulfoxide (DMSO; [?]0.5% [v/v]). Positive control contained a chemical inhibitor selective for each CYP enzyme in the presence of PC945 or posaconazole.

2.4. *In vitro* plasma protein binding

Plasma protein binding of PC945 was determined by ultrafiltration in pooled plasma samples from human, dog, rat and mouse. PC945 was tested at 0.1, 1, 5, 25 and 50 μM in plasma for each species. PC945-containing plasma samples were centrifuged at 45,000 rpm for 20 hours at 37°C. Supernatant was removed and diluted. The concentration of unbound PC945 was determined using LC-MS/MS, from which the extent of protein binding was calculated.

2.5. Clinical trial

2.5.1. Trial design

This PC945 first-in-human clinical trial (ClinicalTrials.gov Identifier: NCT02715570) was a two-part randomised, placebo-controlled study to assess the safety and tolerability of PC945 in both healthy subjects and subjects with mild asthma, conducted at Parexel Early Phase Clinical Unit (Harrow, UK). The study was considered single-blind as the appearance of active and placebo doses were different, however, it was conducted in a double-blind manner (employing separate dosing and assessment teams).

Part one consisted of a single dose escalation study (Cohort 1) and a repeat dose study (Cohort 2) of inhaled PC945 in healthy subjects. Part two comprised a single dose study of inhaled PC945 in subjects with mild asthma (Cohort 3). The protocol and informed consent form were developed according to the International Council for Harmonisation consolidated guideline for Good Clinical Practice and local regulations and were approved by an Independent Ethics Committee. All subjects signed a written informed consent form before enrolment.

In Cohort 1, 11 healthy subjects (three males and eight females; mean age: 48.5 years, mean weight: 65.32 kg, mean body mass index [BMI]: 23.62 kg/m^2), attended the single ascending dose study. Eight

healthy subjects started the study in which two subjects were randomised to each of the four ascending dose treatment sequences (Table 1); two subjects withdrew due to adverse events unrelated to study medication and both were replaced. One of the replacement subjects subsequently withdrew from the study for personal reasons and was replaced by another subject (Figure 1). Overall, six subjects each received three of four doses of PC945 (0.5, 2, 5, and 10 mg) and one dose of placebo.

Nine healthy subjects (four males and five females; mean age: 33.2 years, mean weight: 66.5 kg, mean BMI: 23.53 kg/m^2) whose forced expiratory volume over 1 second (FEV_1) and forced vital capacity (FVC) were [?]80% of predicted values with an FEV_1/FVC ratio >0.7, received once daily doses of PC945 5 mg or placebo for 7 days (Cohort 2, PC945:placebo=2:1).

In Cohort 3, nine subjects with mild asthma (six males and three females; mean age: 37.7 years, mean weight: 82.70 kg, mean BMI: 26.48 kg/m^2) received a single dose of PC945 5 mg or placebo (PC945:placebo=2:1). Subjects with mild asthma needed to demonstrate a methacholine PC_{20} (concentration of inhaled agonist leading to a fall in FEV_1 of [?]20% of personal best) [?]8 mg/mL at screening and an FEV_1>60% of predicted normal value at least 6 hours after the last use of a short acting β-agonist.

Subjects returned to the study unit for a final follow-up visit 10 days after the last administration of study medication.

2.5.2. Sample collection and handling

Serial blood samples were collected to determine plasma PC945 concentrations. For the single dose study in healthy subjects and in subjects with mild asthma, blood samples were collected pre-dose and at 0.25, 0.5, 1, 2, 4, 6, 8, 24, 30 and 48 hours post-end of inhalation and at the follow-up visit. For the repeat dose study, blood samples were collected (i) pre-dose and at 0.25, 0.5, 1, 2, 4, 6, 8, 10, 12 and 24 hours post-end of inhalation on Days 1 and 7, and 30 and 48 hours on Day 7; (ii) pre-dose on Days 5 and 6; (iii) at the follow-up visit.

2.5.3. Safety assessment

Safety was evaluated based on assessments of adverse events, physical examination, vital signs, 12-lead electrocardiogram, spirometry and clinical laboratory tests. The verbatim terms used to identify adverse events were coded using the Medical Dictionary for Regulatory Activities (version 20.1). All adverse events were mapped to system organ class and preferred term.

Spirometry parameters (FEV_1 and FVC) were measured in all subjects to evaluate the local tolerability and potential for bronchospasm of PC945 in accordance with American Thoracic Society/European Respiratory Society guidelines. Predicted values were calculated using National Health and Nutrition Examination Survey reference equations.[18,19]

2.6. Plasma sample analysis

PC945 concentrations were determined by a validated, specific and sensitive LC-MS/MS method under the supervision of LGC Ltd (Fordham, UK) after the drug was extracted from plasma samples. Lower limit of quantification of the assay was 10 pg/mL for clinical samples and 100 pg/mL for rat and dog plasma samples.

2.7. Pharmacokinetic analysis

Plasma pharmacokinetic parameters of PC945 were estimated using a fully validated version of Phoenix WinNonlin® (version 8.0; Certara, NJ, USA). The estimated pharmacokinetic parameters included the maximum observed concentration (C_{max}), time to C_{max} (t_{max}), the area under the plasma concentration

versus time curve (AUC) from 0 to 24 hours post-dose ($AUC_{0–24h}$), the apparent terminal half-life ($t_{1/2}$) and the accumulation ratio (R_o).

Attainment of steady state was assessed by visual inspection of the pre-dose plots during the 7-day treatment period in human subjects.

2.8. Analysis of lung samples

Portions of rat lung tissue were homogenised in methanol:water (50:50; 15 mL for 1 g tissue). PC945 concentrations in tissue homogenates were analysed using LC-MS/MS. A control rat lung sample (B&K Universal Ltd, Hull, UK) was used as standards and quality control.

3. RESULTS

3.1. Nonclinical toxicokinetic data

3.1.1. Single dose toxicokinetic studies in rats and dogs

Following inhalation of PC945, the increase in systemic exposure was less than proportional at doses of 2.0, 7.4 and 16.6 mg/kg. Systemic exposure to PC945 was consistently higher in female than in male rats (Table 2). C_{max} occurred 6 hours after the start of dosing and PC945 was detectable throughout the 24-hour sampling period after dosing.

The magnitude of exposure obtained in dogs was lower than that in rats at similar dose/kg levels. There was a slightly less than dose-proportional increase in systemic exposure at inhaled PC945 doses of 1.7, 5.3 and 18.1 mg/kg in dogs, and no notable differences in mean values of C_{max} and $AUC_{0\text{-}24h}$ between male and female dogs (Table 3). C_{max} was reached in the samples taken immediately after the 2-hour dosing period; exposure extended for the full 24-hour sampling period at all doses.

3.1.2. Repeat dose toxicokinetic studies in rats and dogs

In both test species, daily systemic exposure was higher on Day 14 than Day 1 following inhaled dosing with PC945 suspension. In rats the R_o values for C_{max} were similar to those for $AUC_{0–24h}$ (ranging from 1.8- to 2.8-fold) and showed no sex dependence (Table 4). There was a trend towards greater accumulation in the highest dose group tested. In dogs, the R_o values for C_{max} were lower than those for $AUC_{0\text{-}24h}$ (ranging from 1.3- to 2.3-fold) on Day 14 (Table 5).

Determination of lung exposure in rats on Day 15 confirmed that the concentration of PC945 in lung samples increased with the daily dose administered. PC945 concentrations in the lung were substantially higher (>2000-fold) than those detected in plasma at all dose levels. There was no clear sex effect in the magnitude of PC945 concentrations in the Day-14 lung tissue samples; however, the ratio of lung to plasma PC945 concentration was consistently lower in female rats, reflecting the sex effect of apparent clearance for PC945 in rats (Table 6).

3.2. *In vitro* drug-drug interaction

The only cytochrome P450 inhibitory interaction observed for PC945 was that on CYP3A4/5 substrates. Without pre-incubation, PC945 showed IC_{50} values of 1.33 μM and 0.085 μM for testosterone and midazolam, respectively. The control CYP3A4/5 inhibitor posaconazole showed differences to PC945 in both inhibitory potency and specificity, with IC_{50} values of 0.081 μM and 0.32 μM for testosterone and midazolam, respectively.

Following a 30-minute pre-incubation, PC945 demonstrated a 5-fold shift in potency for CYP3A4/5 (IC_{50} values: 0.247 μM [i.e., 168 ng/mL; testosterone] and 0.017 μM [i.e., 11.6 ng/mL; midazolam]). PC945 had no time-dependent effect on other CYP isoforms.

3.3. *In vitro* plasma protein binding

PC945 was highly bound to plasma proteins in all species and the extent of binding was independent of the concentration over a range of 0.1 μM to 50 μM. Mean extent of protein binding was between 96% and 98% in human, mouse and rat plasma and 91% in dog plasma (Table 7).

3.4. Clinical study data

3.4.1. Safety and tolerability

PC945 was well tolerated following single doses up to 10 mg and repeat doses of 5 mg daily for 7 days in healthy subjects, and at a single dose of 5 mg in subjects with mild asthma. Escalation of single doses of PC945 from 0.5 to 10 mg was not associated with an increase in treatment-emergent adverse events. All treatment-emergent adverse events were either mild or moderate in intensity and resolved before the end of the study. There were no deaths or serious adverse events in the study. Drug-related treatment-emergent adverse events are summarised in Table 8. In total, seven adverse events required intervention; only one of these was considered drug-related (headache, following a single 10 mg dose of PC945).

No clinically significant changes in lung function were observed in healthy subjects. Importantly, no evidence of acute bronchospasm or significant change in lung function (defined as >15% change from baseline) was observed in any subject with mild asthma who received PC945. One subject had a transient reduction in FEV_1 values >15% compared with baseline 10 minutes after receiving a dose of placebo.

There were no notable differences in mean laboratory values, vital signs, electrocardiogram results or spirometry values (Figure 2) between the placebo and PC945 groups. No clinically significant abnormal laboratory results considered to be related to study drug were reported in any cohort.

3.4.2. Pharmacokinetics

3.4.2.1. Single dose in healthy subjects (Cohort 1)

Following single inhaled administration of PC945 at 0.5, 2, 5 and 10 mg in healthy subjects, geometric mean C_{max} values of 54.7, 128, 322 and 619 pg/mL in plasma were achieved at 1, 4, 5 and 2 hours (median t_{max}) post-end of inhalation, respectively (Table 9 and Figure 3). Geometric mean $t_{\frac{1}{2}}$ ranged from 27.9 to 110 hours over the entire dose range. However, the period over which estimated $t_{\frac{1}{2}}$ was calculated was less than 2-fold the $t_{\frac{1}{2}}$ itself and the area extrapolated was greater than 20% in most cases; estimated $t_{\frac{1}{2}}$ was therefore considered to be unreliable at each dose level. Between-subject variability in the extent of systemic exposure to PC945 at 0.5, 2, 5 and 10 mg was moderate to high, as revealed by geometric coefficient of variations (CVs) for C_{max} and $AUC_{0–24h}$ranging from 39.1% to 107%. Systemic exposure to PC945 in female subjects was not appreciably different to that in male subjects.

3.4.2.2. Single dose in subjects with mild asthma (Cohort 3)

Following single inhaled administration of PC945 at 5 mg, a geometric mean C_{max} of 335 pg/mL in plasma was achieved 4 hours (median t_{max}) post-dose (Figure 4). Between-subject variability in the extent of systemic exposure to PC945 was moderate to high, with geometric CVs for C_{max} and $AUC_{0–24h}$ of 68.7% and 49.7%, respectively. Systemic exposure to PC945 (C_{max} and $AUC_{0–24h}$) in subjects with mild asthma (335 pg/mL and 4950 pg*h/mL, respectively) was not meaningfully different from that in healthy subjects at 5 mg (322 pg/mL and 5440 pg*h/mL, respectively).

3.4.2.3. Repeat dose in healthy subjects (Cohort 2)

Following a single inhaled administration of PC945 5 mg on Day 1, a geometric mean C_{max} of 462 pg/mL in plasma was achieved 2 hours (median t_{max}) post-end of inhalation (Table 10 and Figure 3).

Following repeat daily inhaled administration of PC945 5 mg, a geometric mean C_{max} of 951 pg/mL was achieved at 45 minutes post-end of inhalation on Day 7. Systemic exposure to PC945 ($AUC_{0–24h}$) increased 2.6-fold following 7-day dosing; visual inspection of pre-dose concentrations indicated that steady state had not been reached by Day 7 (Figure 5). Between-subject variability in the extent of systemic drug exposure was moderate with geometric CVs for C_{max} and $AUC_{0–24h}$ ranging from 58.7% to 60.9%.

3.5. Dose proportionality

Systemic exposure to PC945 (C_{max} and $AUC_{0–24h}$) increased with escalating doses from 0.5 to 10 mg in an approximately dose-proportional manner. For a doubling in dose, C_{max} and $AUC_{0–24h}$ values increased 1.77-fold and 1.88-fold,; the 90% confidence interval was within the prescribed limits of 1.6 to 2.5 for $AUC_{0–24h}$(values 1.64, 2.15) indicating dose proportionality, though just outside the lower limit for C_{max} (values 1.54, 2.02)

4. DISCUSSION

PC945 is a novel antifungal triazole, designed specifically for inhaled administration with physico-chemical properties for sustained lung retention and persistent antifungal activity.[13] Drug delivery directly to the lung to treat pulmonary disease is well established.[20,21] This dosing route maximises local efficacy in the lungs whilst minimising the potential for adverse systemic effects or drug interactions. Administration of a drug solution into the lung leads to rapid absorption into the systemic circulation; consequently strategies for developing effective inhaled medicines have focused on lipophilic compounds that have low aqueous solubility and slow dissolution rates in aqueous media.[22] Such compounds are typically delivered to the lungs in a micronized, crystalline form as either an aqueous suspension, a pressurised metered dose inhaler or a dry powder blended with a carrier such as lactose. The slow dissolution of inhaled drug particles enhances local action by prolonging lung retention, whilst simultaneously delaying the rate of delivery to the systemic circulation. This strategy was adopted in the development of PC945.[13]

Following single inhaled doses in rats (Day 1 data), PC945 showed a slow and sustained period of absorption into the systemic circulation such that C_{max} occurred 6 hours after the start of dosing. Similarly, in dogs, there was a slow rate of PC945 absorption from the lung followed by sustained plasma levels that extended beyond 24 hours after the initial dose. Systemic exposure was approximately dose-proportional in both test species. The slow rate of PC945 absorption indicated that accumulation of PC945 would occur with repeat daily dosing in rats and dogs. Repeat dose studies confirmed that dose absorption from the lungs was a rate-limiting process in the pharmacokinetic profile of inhaled PC945.

The pharmacokinetic profile of PC945 in humans showed similar drug behaviour to that observed in the nonclinical species investigated. Systemic exposure to PC945 in humans (C_{max} and $AUC_{0–24h}$) increased with escalating doses of 0.5 to 10 mg in a dose-proportional manner. Following 7-day, once daily dosing with 5 mg PC945, $AUC_{0–24h}$ increased 2.6-fold. Visual inspection of pre-dose plasma concentrations indicated that steady state had not been reached by Day 7, and was predicted to be attained approximately 5 weeks after once daily dosing. The prolonged plasma $t_{1/2}$ (from 37.3 hours on Day 1 to 132 hours on Day 7) was consistent with slow absorption from the lung, demonstrating that a typical lung-dominant process was controlling systemic kinetic behaviour. A rapid t_{max} of 45 minutes on Day 7 was observed in healthy subjects who received 5 mg PC945 once daily, suggesting rapid exposure to the respiratory epithelium.

In rat lung samples on Day 15 after 14-day inhaled dosing, mean concentrations of PC945 were approximately proportional to daily doses and >2000-fold (range 2340 to 5020) higher than the concentrations in plasma. The pharmacokinetic data obtained in rats and dogs indicate that the rates of dissolution and subsequent absorption of PC945 from the lungs are the principal factors determining systemic exposure to PC945

following inhaled dosing. Based on the mean lung:plasma ratio observed in male rats (˜4,300 fold) lung concentrations of PC945 in humans following a 5 mg single dose were estimated to be in the region of the 90% of the minimal inhibitory concentration (MIC_{90}) for clinical strains of *A. fumigatus* (1µg/mL). [13]

One potential factor affecting distribution and clearance of PC945 in the lungs is uptake into macrophages in the alveolar space and/or epithelial cells. Several studies in humans investigating the intra-pulmonary pharmacokinetics of azole antifungal agents, such as itraconazole, voriconazole and posaconazole, have been reported.[23–26] For itraconazole and posaconazole, preferential distribution into alveolar cells could be a favourable factor for successful treatment and prevention of respiratory fungal disease. For example, 14-day oral administration of posaconazole (400 mg twice daily) resulted in steady state in which mean concentrations were over 30-fold greater in pulmonary alveolar cells than in lung epithelial lining fluid and plasma, with an alveolar cell:plasma ratio of 27 to 44 after the last dose.[25] Similar pulmonary pharmacokinetic data have been reported with oral posaconazole treatment in lung transplant patients, suggesting that uptake into alveolar cells is a reproducible distribution characteristic of the compound in clinical studies.[26] Preferential uptake of antifungal agents into alveolar cells (which are principally macrophages) could be clinically relevant, as macrophages are a host defence mechanism against alveolar infection by *Aspergillus* and scavenge to remove particulate matter.[7,27]

It is well known that all antifungal azoles are potent inhibitors of cytochrome P450 (CYP450) enzymes, leading to a significant risk of interactions with other co-medicated drugs.[7,28] All of these agents potently inhibit human CYP3A4 isoforms and the inhibitory activity is extended to human CYP2C9 and CYP2C19 in the cases of fluconazole and voriconazole.[7] It is also reported that posaconazole has the potential to inhibit CYP450-mediated steroid hormone synthesis causing hypertrophy or hyperplasia of the adrenal glands.[29] These CYP inhibitory actions are well established as a mechanism of pharmacokinetics-based drug-drug interactions in the clinic.[7] PC945 is a potent inhibitor of human CYP3A4/5. However, oral inhalation of PC945 resulted in low systemic exposure as demonstrated by a mean plasma C_{max} of 951 pg/mL [equivalent to 0.0016 µM] following 7-day, once daily, 5 mg doses, an order of magnitude lower than the inhibitory IC_{50} value (0.017 µM; determined as the time-dependent value for *in vitro* inhibition of midazolam in pooled human liver microsomes). Moreover, the C_{max}determined for PC945 [0.0016 µM] is much lower than that of either itraconazole (1.52 µM after 15-day, 200 mg daily oral dosing) or posaconazole (0.83 µM after 200 mg three times daily oral dosing).[30,31] The low C_{max}/IC_{50} ratio of 0.1 for PC945 indicates that a systemic based, CYP3A4/5 mediated, hepatic drug-drug interaction is unlikely.

In the first-in-human study, reported herein, PC945 was well tolerated in healthy subjects at either single doses up to 10 mg or at repeat doses of 5 mg once daily for 7 days in addition to subjects with mild asthma at a single dose of 5 mg. No evidence of irritancy was observed in subjects with mild asthma.

In summary, PC945 was well tolerated at all doses tested and no safety signals were identified. The pharmacokinetic profile translated well from nonclinical species to results derived from the clinic. Key findings of the study reveal that PC945 undergoes slow absorption from the lung and exhibits low systemic exposure thus limiting the potential for an adverse side effect profile and drug interactions commonly seen with oral azoles. In addition, systemic exposure to PC945 in subjects with mild asthma showed no appreciable difference in its profile to that observed in healthy subjects at the same dose level, suggesting no dose adjustment would be anticipated in subsequent studies in patients. We demonstrated that 5 mg is a suitable dose and the data, together with a case report describing the treatment of fungal tracheobronchitis post lung transplantation, support the further development of PC945 in studies of patients with pulmonary aspergillosis.[32]

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CONFLICT OF INTERESTS

All authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author). Pulmocide Ltd. provided funding for this clinical trial. LC, AM and AD are employees of Pulmocide Ltd and own stock options of Pulmocide Ltd. KI and PS are (co)founders and employees of Pulmocide Ltd, and own stock options of Pulmocide Ltd. JM and GR are (co)founders and consultants of Pulmocide Ltd. and own stocks of Pulmocide Ltd. KW, JA, JP, EF, PH and CW (are part-time consultants to Pulmocide Ltd)

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request. REFERENCES

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TABLES

Table 1 Treatment sequences for the single ascending dose study (Cohort 1)

Week 1[a,b]
PC945 0.5 mg
PC945 0.5 mg
PC945 0.5 mg
Placebo
[a] Sentinel dosing: two subjects (PC945:placebo=1:1) were dosed on Day 1; the remaining six subjects were dosed on Day 2

Table 2 Mean systemic exposure[a] to PC945 in male and female rats following single inhaled doses

Dose (mg/kg)	**Male rats**	**Male rats**	**Female rats**	**Female rats**
(ng/mL)	**C_{max}** **$AUC_{0\text{-}24h}$ (ng·h/mL)**	**C_{max}**		
(ng/mL)	**$AUC_{0\text{-}24h}$ (ng·h/mL)**			
2.0	7.3	103	10.5	183
7.1	15.8	217	24.8	416
16.6	26.0	426	41.3	731
Abbreviations: C_{max}, maximum observed concentration; $AUC_{0\text{-}24h}$, area under the plasma concentration curve from time zero to 24 hours post-dose. [a] No standard deviations were determined on pharmacokinetic parameters as these were derived from PC945 concentrations in groups of rats per time point.	Abbreviations: C_{max}, maximum observed concentration; $AUC_{0\text{-}24h}$, area under the plasma concentration curve from time zero to 24 hours post-dose. [a] No standard deviations were determined on pharmacokinetic parameters as these were derived from PC945 concentrations in groups of rats per time point.	Abbreviations: C_{max}, maximum observed concentration; $AUC_{0\text{-}24h}$, area under the plasma concentration curve from time zero to 24 hours post-dose. [a] No standard deviations were determined on pharmacokinetic parameters as these were derived from PC945 concentrations in groups of rats per time point.	Abbreviations: C_{max}, maximum observed concentration; $AUC_{0\text{-}24h}$, area under the plasma concentration curve from time zero to 24 hours post-dose. [a] No standard deviations were determined on pharmacokinetic parameters as these were derived from PC945 concentrations in groups of rats per time point.	Abbreviations: C_{max}, maximum observed concentration; $AUC_{0\text{-}24h}$, area under the plasma concentration curve from time zero to 24 hours post-dose. [a] No standard deviations were determined on pharmacokinetic parameters as these were derived from PC945 concentrations in groups of rats per time point.

Table 3 Mean (standard deviation) systemic exposure to PC945 in male and female dogs following a single inhaled dose

Dose (mg/kg)	**Male dogs**	**Male dogs**	**Female dogs**	**Female dogs**
	C_{max} (ng/mL)	**$AUC_{0\text{-}24h}$ (ng·h/mL)**	**C_{max} (ng/mL)**	**$AUC_{0\text{-}24h}$ (ng·h/mL)**
1.7	2.2 ± 0.3	17.2 ± 3.5	1.5 ± 0.5	12.2 ± 2.9
5.3	4.6 ± 0.4	38.5 ± 1.8	4.3 ± 0.7	39.0 ± 2.7
18.1	9.7 ± 2.4	88.5 ± 25.8	10.2 ± 1.3	90.6 ± 6.7
Abbreviations: C_{max}, maximum observed concentration; $AUC_{0\text{-}24h}$, area under the plasma concentration curve from time zero to 24 hours post-dose.	Abbreviations: C_{max}, maximum observed concentration; $AUC_{0\text{-}24h}$, area under the plasma concentration curve from time zero to 24 hours post-dose.	Abbreviations: C_{max}, maximum observed concentration; $AUC_{0\text{-}24h}$, area under the plasma concentration curve from time zero to 24 hours post-dose.	Abbreviations: C_{max}, maximum observed concentration; $AUC_{0\text{-}24h}$, area under the plasma concentration curve from time zero to 24 hours post-dose.	Abbreviations: C_{max}, maximum observed concentration; $AUC_{0\text{-}24h}$, area under the plasma concentration curve from time zero to 24 hours post-dose.

Table 4 Summary of systemic exposure[a] to PC945 in male and female rats following 14 days of inhaled dosing of 2.0, 7.1 and 16.6 mg/kg/day

Dose (mg/kg)	**Male rats**	**Male rats**	**Male rats**	**Female rats**	**Female rats**	**Female rats**
	C_{max}					
(ng/mL)	**$AUC_{0\text{-}24h}$**					
(ng·h/mL)	**R_o[b]**	**C_{max}**				
(ng/mL)	**$AUC_{0\text{-}24h}$**					
(ng·h/mL)	**R_o[b]**					
2.0	12.3	202	1.96	19.2	336	1.84
7.1	23.7	424	1.91	50.4	749	1.91
16.6	76.9	1140	2.76	92.3	1850	2.53

Dose (mg/kg)	**Male rats**	**Male rats**	**Male rats**	**Female rats**	**Female rats**	**Female rats**
Abbreviations: C_{max}, maximum observed concentration; $AUC_{0\text{-}24h}$, area under the plasma concentration curve from time zero to 24 hours post-dose; R_o, accumulation ratio. [a] No standard deviation on pharmacokinetic parameters as these were derived from PC945 concentrations in groups of rats per time point. [b] Accumulation ratio (R_o) derived from $AUC_{0\text{-}24h}$ (Day14) / $AUC_{0\text{-}24h}$ (Day 1). Day 1 data are shown in Table 2.	Abbreviations: C_{max}, maximum observed concentration; $AUC_{0\text{-}24h}$, area under the plasma concentration curve from time zero to 24 hours post-dose; R_o, accumulation ratio. [a] No standard deviation on pharmacokinetic parameters as these were derived from PC945 concentrations in groups of rats per time point. [b] Accumulation ratio (R_o) derived from $AUC_{0\text{-}24h}$ (Day14) / $AUC_{0\text{-}24h}$ (Day 1). Day 1 data are shown in Table 2.	Abbreviations: C_{max}, maximum observed concentration; $AUC_{0\text{-}24h}$, area under the plasma concentration curve from time zero to 24 hours post-dose; R_o, accumulation ratio. [a] No standard deviation on pharmacokinetic parameters as these were derived from PC945 concentrations in groups of rats per time point. [b] Accumulation ratio (R_o) derived from $AUC_{0\text{-}24h}$ (Day14) / $AUC_{0\text{-}24h}$ (Day 1). Day 1 data are shown in Table 2.	Abbreviations: C_{max}, maximum observed concentration; $AUC_{0\text{-}24h}$, area under the plasma concentration curve from time zero to 24 hours post-dose; R_o, accumulation ratio. [a] No standard deviation on pharmacokinetic parameters as these were derived from PC945 concentrations in groups of rats per time point. [b] Accumulation ratio (R_o) derived from $AUC_{0\text{-}24h}$ (Day14) / $AUC_{0\text{-}24h}$ (Day 1). Day 1 data are shown in Table 2.	Abbreviations: C_{max}, maximum observed concentration; $AUC_{0\text{-}24h}$, area under the plasma concentration curve from time zero to 24 hours post-dose; R_o, accumulation ratio. [a] No standard deviation on pharmacokinetic parameters as these were derived from PC945 concentrations in groups of rats per time point. [b] Accumulation ratio (R_o) derived from $AUC_{0\text{-}24h}$ (Day14) / $AUC_{0\text{-}24h}$ (Day 1). Day 1 data are shown in Table 2.	Abbreviations: C_{max}, maximum observed concentration; $AUC_{0\text{-}24h}$, area under the plasma concentration curve from time zero to 24 hours post-dose; R_o, accumulation ratio. [a] No standard deviation on pharmacokinetic parameters as these were derived from PC945 concentrations in groups of rats per time point. [b] Accumulation ratio (R_o) derived from $AUC_{0\text{-}24h}$ (Day14) / $AUC_{0\text{-}24h}$ (Day 1). Day 1 data are shown in Table 2.	Abbreviations: C_{max}, maximum observed concentration; $AUC_{0\text{-}24h}$, area under the plasma concentration curve from time zero to 24 hours post-dose; R_o, accumulation ratio. [a] No standard deviation on pharmacokinetic parameters as these were derived from PC945 concentrations in groups of rats per time point. [b] Accumulation ratio (R_o) derived from $AUC_{0\text{-}24h}$ (Day14) / $AUC_{0\text{-}24h}$ (Day 1). Day 1 data are shown in Table 2.

Table 5 Summary of mean[a] systemic exposure to PC945 in male and female dogs following 14 days of inhaled dosing of 1.7, 5.3 and 18.1 mg/kg/day

Dose

(mg/kg)

Male dogs

Male dogs	
Male dogs	
Female dogs	
Female dogs	
Female dogs	
C_{max} (ng/mL)	
$AUC_{0\text{-}24h}$ (ng·h/mL)	
R_o[b]	
C_{max} (ng/mL)	
$AUC_{0\text{-}24h}$ (ng·h/mL)	
R_o[b]	
1.7	
2.8	
35.5	
2.06	
2.3	
27.7	
2.27	
5.3	
5.6	
70.9	
1.84	
5.5	
73.3	
1.88	
18.1	
13.5	
203	
2.29	
7.8	
114	
1.26	

Abbreviations: C_{max}, maximum observed concentration; $AUC_{0\text{-}24h}$, area under the plasma concentration curve from time zero to 24 hours post-dose; R_o, accumulation ratio. [a] Standard Deviation values were

omitted for reasons of clarity in presentation of mean data. [b] Accumulation ratio (Ro) was derived from $AUC_{0\text{-}24h}$ (Day14) / $AUC_{0\text{-}24h}$ (Day 1). Day 1 data are shown in Table 3.

Abbreviations: C_{max}, maximum observed concentration; $AUC_{0\text{-}24h}$, area under the plasma concentration curve from time zero to 24 hours post-dose; R_o, accumulation ratio. [a] Standard Deviation values were omitted for reasons of clarity in presentation of mean data. [b] Accumulation ratio (Ro) was derived from $AUC_{0\text{-}24h}$ (Day14) / $AUC_{0\text{-}24h}$ (Day 1). Day 1 data are shown in Table 3.

Abbreviations: C_{max}, maximum observed concentration; $AUC_{0\text{-}24h}$, area under the plasma concentration curve from time zero to 24 hours post-dose; R_o, accumulation ratio. [a] Standard Deviation values were omitted for reasons of clarity in presentation of mean data. [b] Accumulation ratio (Ro) was derived from $AUC_{0\text{-}24h}$ (Day14) / $AUC_{0\text{-}24h}$ (Day 1). Day 1 data are shown in Table 3.

Abbreviations: C_{max}, maximum observed concentration; $AUC_{0\text{-}24h}$, area under the plasma concentration curve from time zero to 24 hours post-dose; R_o, accumulation ratio. [a] Standard Deviation values were omitted for reasons of clarity in presentation of mean data. [b] Accumulation ratio (Ro) was derived from $AUC_{0\text{-}24h}$ (Day14) / $AUC_{0\text{-}24h}$ (Day 1). Day 1 data are shown in Table 3.

Abbreviations: C_{max}, maximum observed concentration; $AUC_{0\text{-}24h}$, area under the plasma concentration curve from time zero to 24 hours post-dose; R_o, accumulation ratio. [a] Standard Deviation values were omitted for reasons of clarity in presentation of mean data. [b] Accumulation ratio (Ro) was derived from $AUC_{0\text{-}24h}$ (Day14) / $AUC_{0\text{-}24h}$ (Day 1). Day 1 data are shown in Table 3.

Abbreviations: C_{max}, maximum observed concentration; $AUC_{0\text{-}24h}$, area under the plasma concentration curve from time zero to 24 hours post-dose; R_o, accumulation ratio. [a] Standard Deviation values were omitted for reasons of clarity in presentation of mean data. [b] Accumulation ratio (Ro) was derived from $AUC_{0\text{-}24h}$ (Day14) / $AUC_{0\text{-}24h}$ (Day 1). Day 1 data are shown in Table 3.

Abbreviations: C_{max}, maximum observed concentration; $AUC_{0\text{-}24h}$, area under the plasma concentration curve from time zero to 24 hours post-dose; R_o, accumulation ratio. [a] Standard Deviation values were omitted for reasons of clarity in presentation of mean data. [b] Accumulation ratio (Ro) was derived from $AUC_{0\text{-}24h}$ (Day14) / $AUC_{0\text{-}24h}$ (Day 1). Day 1 data are shown in Table 3.

Table 6 Summary of mean plasma C_{max} and mean lung tissue concentrations of PC945 in male and female rats following 14 days of inhaled dosing of 2.0, 7.1 and 16.6 mg/kg/day

Achieved Inhaled Dose (mg/kg)
2.0
7.1
16.6

Abbreviation: C_{max}, maximum observed concentration. [a] Ratio was derived from lung tissue PC945 concentration (Day 15)

Table 7 Summary of mean and range of cross-species plasma protein binding

Species

Mean percentage

protein bound[a]

Range of % bound values across concentration range

Human

96.6

94.7–98.6

Dog

91.1

82.3–97.2

Rat

97.8

96.8–98.3

Mouse

96.2

93.0–98.7

[a] Mean values for protein binding cover the PC945 range of 0.1 μM to 50 μM. No clear trend of concentration dependent binding was observed.

Table 8 Summary of subjects with treatment-emergent adverse events considered by investigators to be related to study drug (First-in-Human Study)

	Cohort 1 (Single dose) n (%)	**Cohort 1 (Single dose) n (%)**	**Cohort 1 (Single dose) n (%)**	**Cohort 1 (Single dose) n (%)**	**Cohort 1 (Single dose) n (%)**	**Cohort 2 (Once daily for 7 days) n (%)**	**Cohort 2 (Once daily for 7 days) n (%)**	**Cohort 3 (Single dose) n (%)**
	Placebo[a]	**PC945 0.5 mg**	**PC945 2 mg**	**PC945 5 mg**	**PC945 10 mg**	**Placebo**	**PC945 5 mg**	**Placebo**
	N=8	**N=6**	**N=6**	**N=6**	**N=6**	**N=3**	**N=6**	**N=3**
No. of subjects with treatment-emergent adverse events attributed to study drug	4 (50.00)	1 (16.67)	0	2 (33.33)	2 (33.33)	1 (33.33)	2 (33.33)	0
Diarrhoea	0	0	0	0	1 (16.7)	0	0	0
Faeces soft	1 (12.50)	0	0	1 (16.67)	0	0	0	0

	Cohort 1 (Single dose) n (%)	**Cohort 1 (Single dose) n (%)**	**Cohort 1 (Single dose) n (%)**	**Cohort 1 (Single dose) n (%)**	**Cohort 1 (Single dose) n (%)**	**Cohort 2 (Once daily for 7 days) n (%)**	**Cohort 2 (Once daily for 7 days) n (%)**	**Cohort 3 (Single dose) n (%)**
Nausea	0	0	0	1 (16.67)	1 (16.67)	0	1 (16.67)	0
Fatigue	0	1 (16.67)	0	0	0	0	0	0
Muscle spasms	0	0	0	1 (16.67)	0	0	0	0
Musculoskeletal discomfort	1 (12.50)	0	0	0	0	0	0	0
Dizziness	0	0	0	1 (16.67)	1 (16.67)	0	0	0
Headache	1 (12.50)	0	0	1 (16.67)	1 (16.67)	0	1 (16.67)	0
Lethargy	0	0	0	1 (16.67)	0	0	0	0
Cough	1 (12.50)	0	0	0	0	0	0	0
Productive cough	1 (12.50)	0	0	0	0	0	0	0
Throat tightness	0	0	0	0	0	1 (33.33)	0	0

	Cohort 1 (Single dose) n (%)	**Cohort 1 (Single dose) n (%)**	**Cohort 1 (Single dose) n (%)**	**Cohort 1 (Single dose) n (%)**	**Cohort 1 (Single dose) n (%)**		**Cohort 2 (Once daily for 7 days) n (%)**	**Cohort 2 (Once daily for 7 days) n (%)**		**Cohort 3 (Single dose) n (%)**
N=number of subjects per group; n=number of subjects with AEs attributed to study drug. Only treatment-emergent adverse events included in this table. All adverse events are coded using medical Dictionary for Regulatory Activities (MedDRA) version 20.1. [a]	N=number of subjects per group; n=number of subjects with AEs attributed to study drug. Only treatment-emergent adverse events included in this table. All adverse events are coded using medical Dictionary for Regulatory Activities (MedDRA) version 20.1. [a]	N=number of subjects per group; n=number of subjects with AEs attributed to study drug. Only treatment-emergent adverse events included in this table. All adverse events are coded using medical Dictionary for Regulatory Activities (MedDRA) version 20.1. [a]	N=number of subjects per group; n=number of subjects with AEs attributed to study drug. Only treatment-emergent adverse events included in this table. All adverse events are coded using medical Dictionary for Regulatory Activities (MedDRA) version 20.1. [a]	N=number of subjects per group; n=number of subjects with AEs attributed to study drug. Only treatment-emergent adverse events included in this table. All adverse events are coded using medical Dictionary for Regulatory Activities (MedDRA) version 20.1. [a]	N=number of subjects per group; n=number of subjects with AEs attributed to study drug. Only treatment-emergent adverse events included in this table. All adverse events are coded using medical Dictionary for Regulatory Activities (MedDRA) version 20.1. [a]	N=number of subjects per group; n=number of subjects with AEs attributed to study drug. Only treatment-emergent adverse events included in this table. All adverse events are coded using medical Dictionary for Regulatory Activities (MedDRA) version 20.1. [a]	N=number of subjects per group; n=number of subjects with AEs attributed to study drug. Only treatment-emergent adverse events included in this table. All adverse events are coded using medical Dictionary for Regulatory Activities (MedDRA) version 20.1. [a]	N=number of subjects per group; n=number of subjects with AEs attributed to study drug. Only treatment-emergent adverse events included in this table. All adverse events are coded using medical Dictionary for Regulatory Activities (MedDRA) version 20.1. [a]	N=number of subjects per group; n=number of subjects with AEs attributed to study drug. Only treatment-emergent adverse events included in this table. All adverse events are coded using medical Dictionary for Regulatory Activities (MedDRA) version 20.1. [a]	N=number of subjects per group; n=number of subjects with AEs attributed to study drug. Only treatment-emergent adverse events included in this table. All adverse events are coded using medical Dictionary for Regulatory Activities (MedDRA) version 20.1. [a]
Placebo was	Placebo was	Placebo was	Placebo was	Placebo was	Placebo was	Placebo was	Placebo was	Placebo was	Placebo was	Placebo was

	Cohort 1 (Single dose) n (%)	**Cohort 1 (Single dose) n (%)**	**Cohort 1 (Single dose) n (%)**	**Cohort 1 (Single dose) n (%)**	**Cohort 1 (Single dose) n (%)**	**Cohort 2 (Once daily for 7 days) n (%)**	**Cohort 2 (Once daily for 7 days) n (%)**	**Cohort 3 (Single dose) n (%)**

Table 9 Geometric mean (CV%) pharmacokinetic parameters of PC945 following single doses of PC945 in healthy subjects (Cohort 1)

Parameter
(N=6)
(N=6)
(N=6)
(N=6)
C_{max} (pg/mL)
t_{max} (h)[a]
$AUC_{0–24h}$ (pg*h/mL)
$AUC_{0–[?]}$ (pg*h/mL)
$t_{\frac{1}{2}}$ (h)
For t_{max}, data are presented as median (range). Abbreviations: CV, coefficient of variation; C_{max}, maximum plasma concen

Table 10 Geometric mean (CV%) pharmacokinetic parameters of PC945 following repeat doses of PC945 5 mg in healthy subjects (Cohort 2)

PK Parameter
C_{max} (pg/mL)
t_{max} (h)
$AUC_{0–24h}$ (pg*h/mL)
$t_{\frac{1}{2}}$ (h)
R_O
For t_{max}, data are presented as median (range). Abbreviations: CV, coefficient of variation; C_{max}, maximum plasma concen

FIGURE LEGENDS

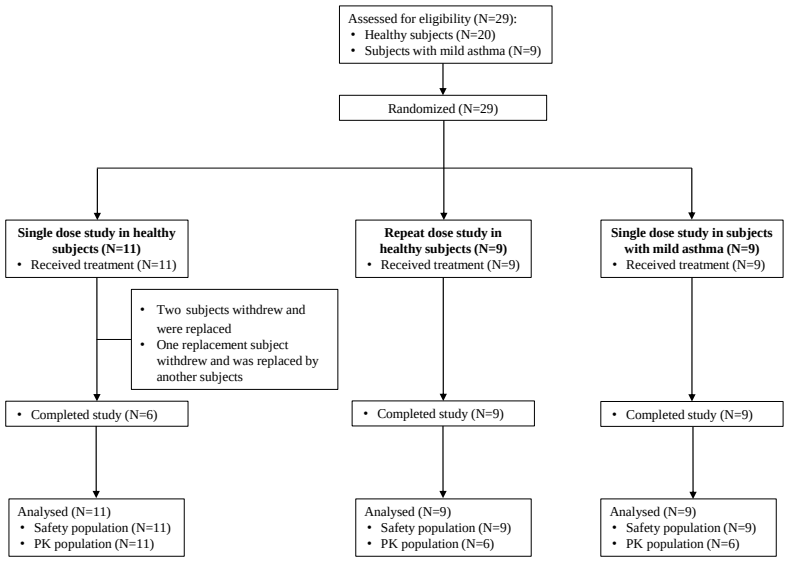
Figure 1 Subject disposition (CONSORT diagram)

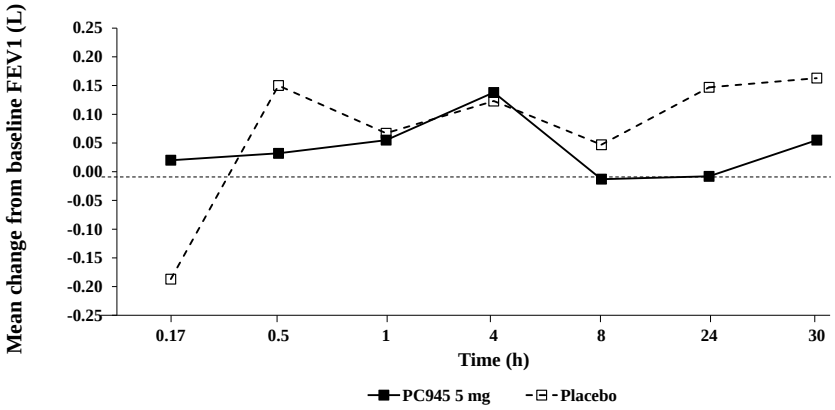
Figure 2 Mean change from baseline in FEV_1 for subjects with mild asthma (Cohort 3)

Figure 3 Mean plasma concentrations (linear [A] and log scales [B]) vs. time profiles of PC945 after single-dose administration in healthy subjects (Cohort 1 and Day 1 of Cohort 2)

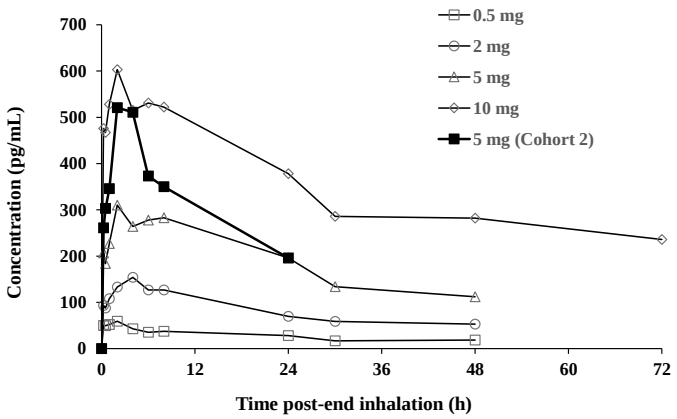
Figure 4 Mean plasma concentrations (linear [A] and log scales [B]) vs. time profiles of PC945 following a single inhalation of PC945 5 mg in healthy subjects (Cohort 1) and subjects with mild asthma (Cohort 3)

Figure 5 Mean plasma concentrations (linear [A] and log scales [B]) vs. time profiles of PC945 following repeat daily administration of PC945 5 mg in healthy subjects (Cohort 2, Day 1 and Day 7)

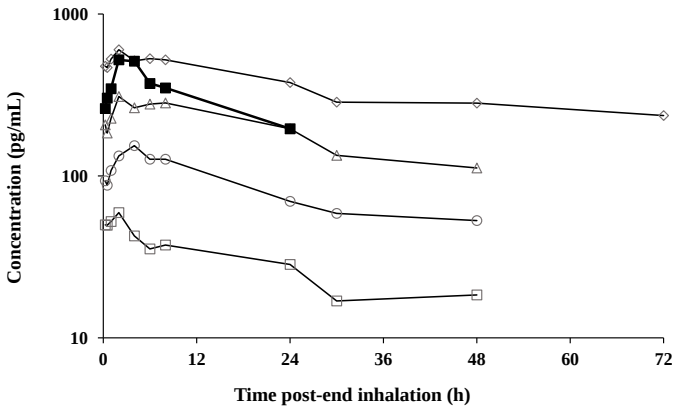




A



B



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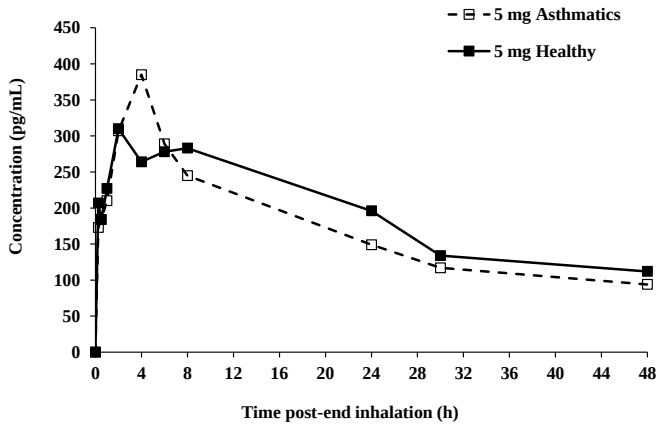

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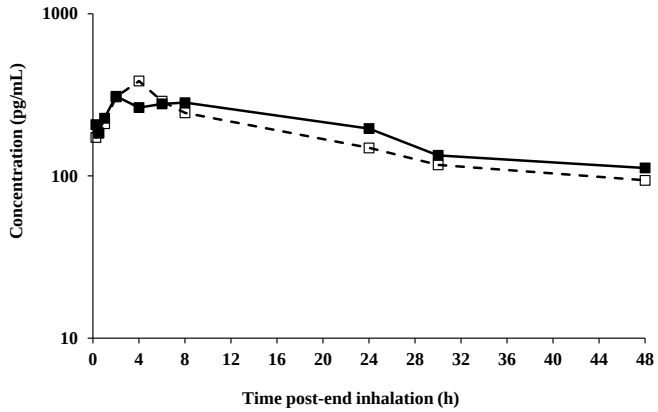

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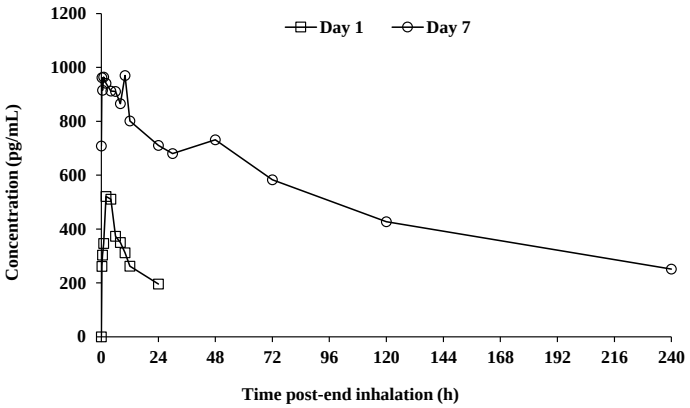

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