

EFFECT OF POL6014, A POTENT AND SELECTIVE INHALED NEUTROPHIL ELASTASE INHIBITOR, IN A RAT MODEL OF LUNG NEUTROPHIL ACTIVATION

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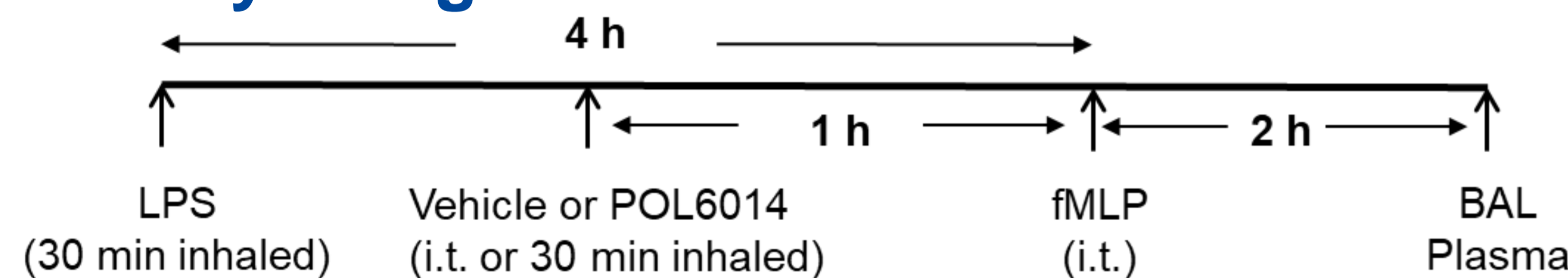
Rationale

Excessive neutrophil elastase (NE) activity caused by chronic neutrophilic inflammation predominantly due to recurrent infection in cystic fibrosis (CF) or non-cystic fibrosis bronchiectasis (NCFB) is a key contributor to deterioration of lung function¹.

POL6014 has been shown to be a potent, reversible, competitive and selective inhibitor of human NE. POL6014, administered directly into the airways has a very favourable pharmacokinetic profile² and was highly effective in animal models of lung inflammation. POL6014 was successfully formulated for aerosol delivery via nebulization by the Pari eFlow system and is currently in clinical development for treatment of Cystic Fibrosis being led by Santhera Pharmaceuticals.

Here the effect of POL6014 was investigated after direct pulmonary delivery in a rat model of lung neutrophil activation induced by sequential administration of lipopolysaccharide (LPS) and N-formyl-methionyl-leucyl-phenylalanine (fMLP).

Study design



Methods

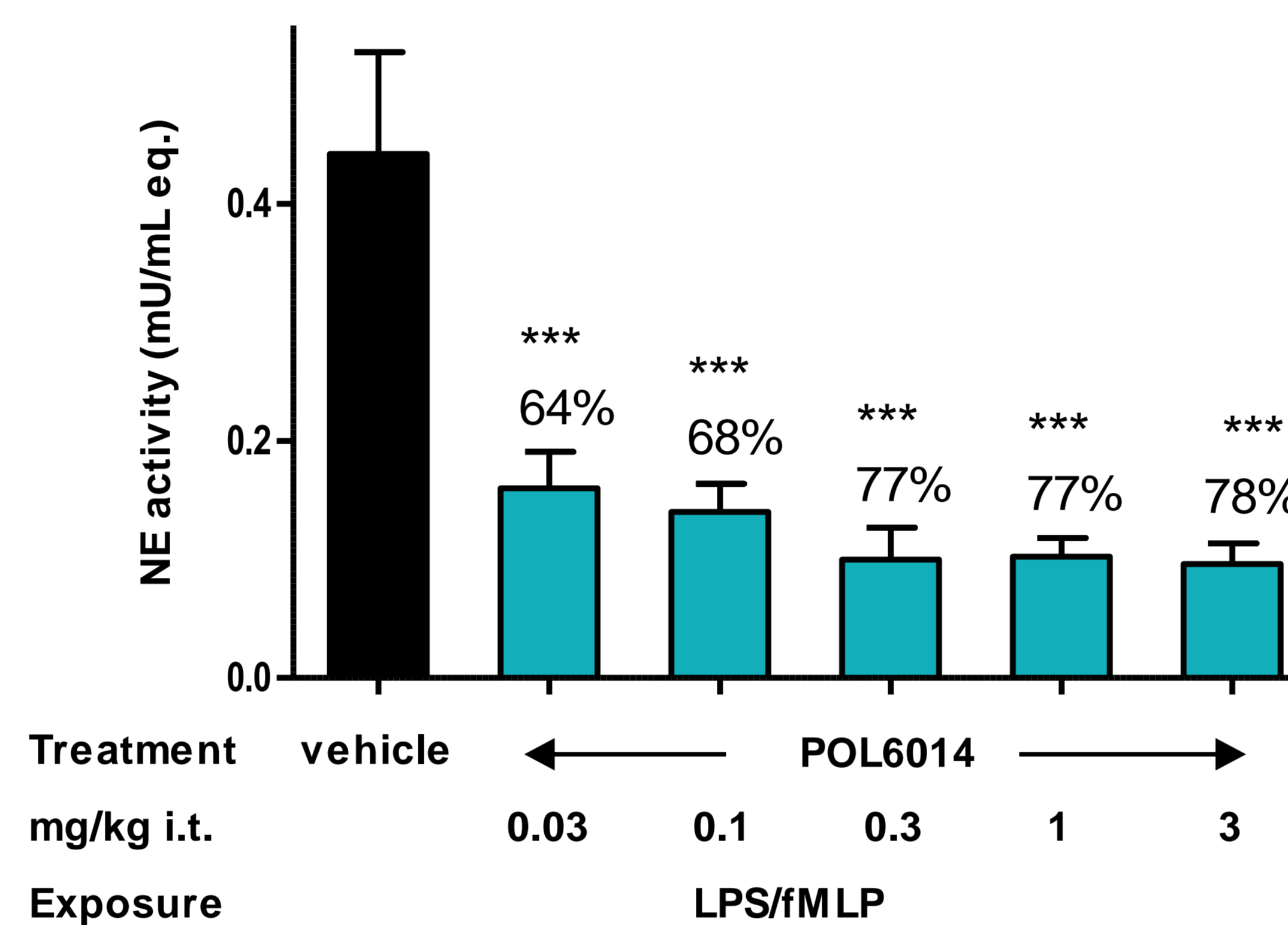
Male Sprague Dawley rats were challenged with aerosolized LPS from *E.coli* serotype O26:B6 (1 mg/mL) for 30 min and dosed with fMLP (5 mg/kg) by intratracheal route (i.t.) 4 h post completion of the LPS challenge. Animals were either treated with POL6014 or vehicle (0.5% NaCl pH 5.5) 1 h prior to fMLP administration, by i.t. or by snout only inhalation for 30 min (lung doses from 0.03 to 3 mg/kg). Animals were terminated 6 h following LPS challenge. Lung samples were collected on satellite animals at the end of the 30 min inhalation of POL6014.

Aerosol was generated with Pari eFlow nebulizer and aerosol concentration was determined by analyzing samples collected on glass fiber filters by HPLC. Particle size distribution was determined by cascade impactor. NE activity was measured in bronchoalveolar lavage (BAL) fluid using the fluorogenic substrate MeOSuc-AAPV-AMC. Fluorescence was then converted into an equivalent concentration human NE (mU/mL) using human NE standard range. Total and differential cell counts in BAL were performed by flow cytometry. The concentration of POL6014 in rat plasma and lung was determined using UHPLC-MS/MS.

All the groups were compared using one way ANOVA followed by Bonferroni's Multiple Comparison Test.

POL6014 i.t. significantly and dose-dependently inhibits BAL NE activity

Figure 1: Effects of POL6014 on BAL NE activity in a LPS/fMLP model by i.t. administration. Values are expressed as mean ± SEM (n=10/group). *** p<0.001 vs vehicle group. The % reduction of NE activity vs vehicle is reported above each bar.



Aerosol characteristics

Figure 2: Schematic of inhalation exposure system for rodents

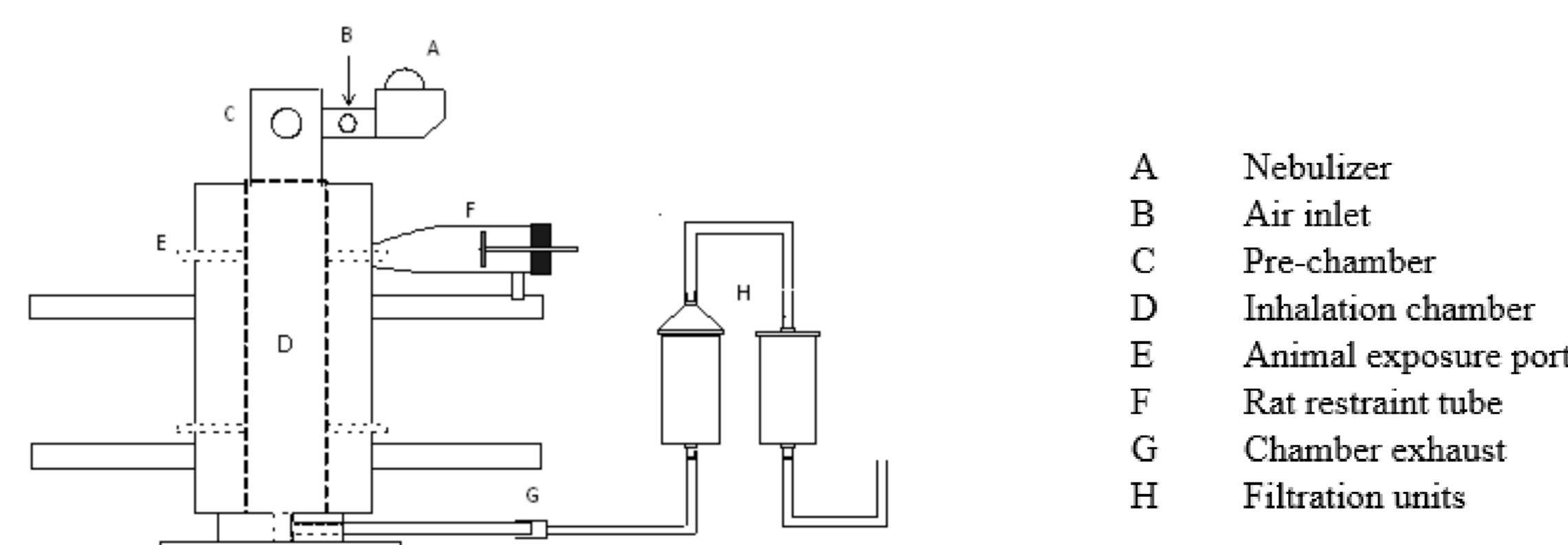


Table 1: Aerosol parameters and estimated lung-deposited doses

^a calculated based on ref 3 and a pulmonary deposition fraction of 10%
^b mass median aerodynamic diameter ± geometric standard deviation

| Group | Aerosol concentration (µg/L) | Estimated Lung dose ^a (mg/kg) | Particle size ^b (µm) |
|-------------|------------------------------|--|---------------------------------|
| 1 (vehicle) | - | - | - |
| 2 | 16.3 | 0.036 | 0.6 ± 2.68 |
| 3 | 210 | 0.457 | 1.7 ± 1.85 |
| 4 | 1730 | 3.76 | 2.2 ± 1.65 |

POL6014 inhaled significantly inhibits BAL NE activity

Figure 3: Effects of inhaled POL6014 on BAL NE activity in a LPS/fMLP model. Values are expressed as mean ± SEM (n=10/group). *** p<0.001 vs vehicle group. The % reduction of NE activity vs vehicle is reported above each bar

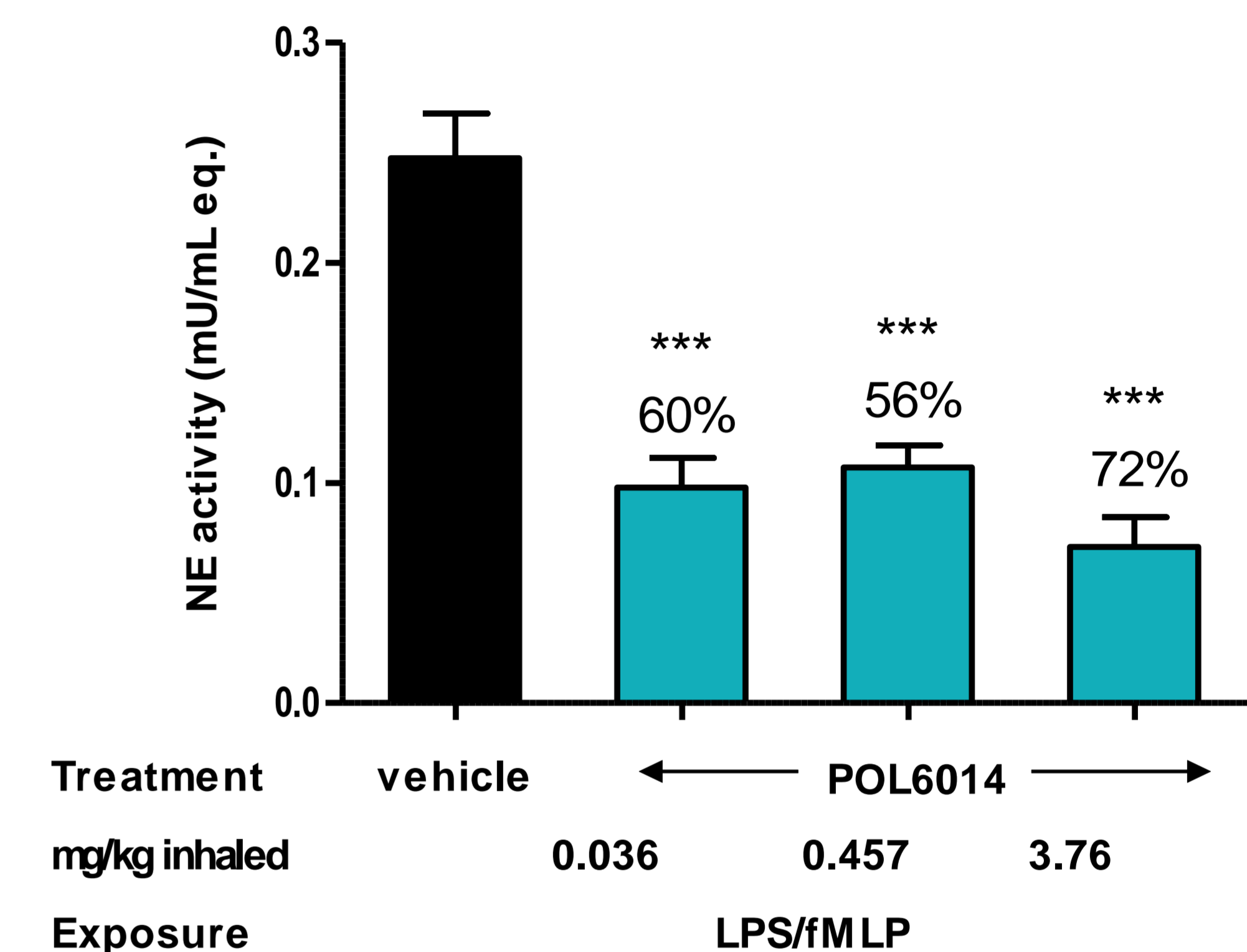
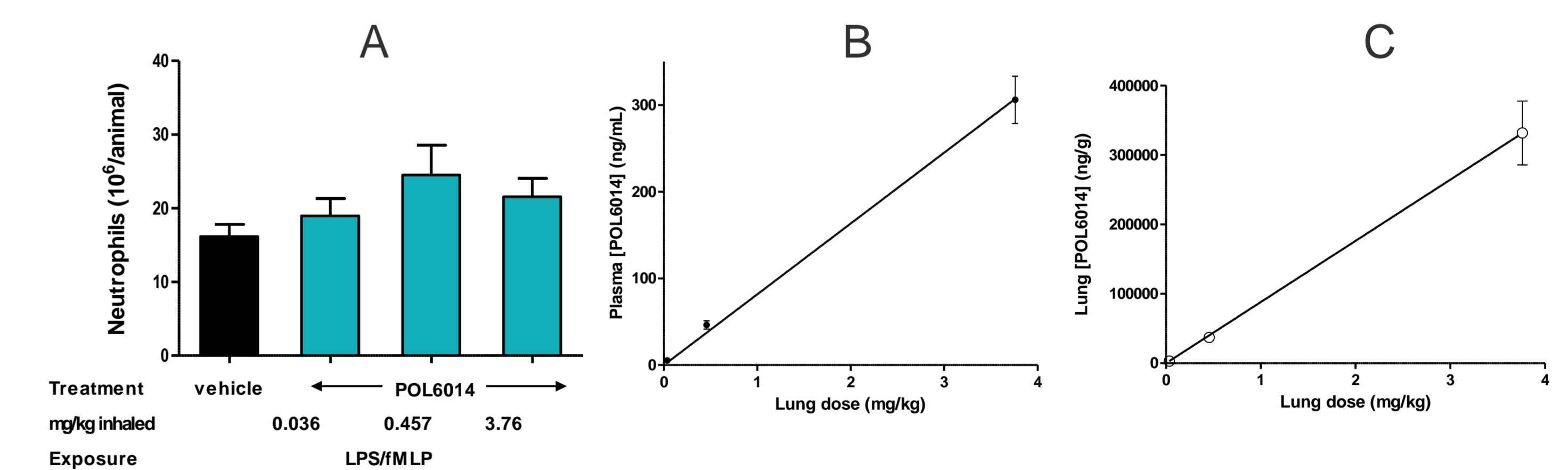


Figure 4: A: BAL neutrophil counts in a LPS/fMLP model. B and C: Plasma levels (3 h post inhalation start) and lung levels (30 min post inhalation start) versus estimated lung dose.



- ❖ No significant effect on neutrophils in BAL was observed.
- ❖ Lung levels of POL6014 are approximately 1000 times higher than those detected in plasma.

Conclusion

- ✓ POL6014 dose-dependently and significantly reduced NE activity in a rat LPS/fMLP model when administered directly into the lung without reducing neutrophil counts.
- ✓ These encouraging results support the development of inhaled POL6014 for reduction of inflammation in neutrophilic lung diseases like CF and NCFB.

References

1. Mayer-Hamblett N et al (2007) Am J Respir Crit Care Med 175:822–828.
2. Chevalier E et al (2009) Am J Respir Crit Care Med 179: A5652
3. Alexander DJ et al (2008) Inhal Toxicol 20:1179–1189