A novel Protein Epitope Mimetic (PEM) neutrophil elastase (NE) inhibitor, POL6014, inhibits human NE-induced acute lung injury in mice.

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Abstract

Rationale: Neutrophil elastase (NE) is described to be involved in the inflammatory processes of lung diseases associated with tissue remodeling including acute lung injury (ALI), cystic fibrosis (CF), chronic obstructive pulmonary disease (COPD) and emphysema. The aim of this study was to investigate the effect of a local instillation of POL6014, a novel reversible PEM NE inhibitor, on human neutrophil elastase (HNE)-induced ALI in mice.
Methods: POL6014 (0.05, 0.2, 0.5 and 5 mg/kg) or ONO-5046 (1 and 5 mg/kg) were administered intranasally (i.n.) 15 minutes before HNE (30 UI, i.n.) in C57BL/6j mice. Cell composition and the levels of hemoglobin (Hb), interleukin (IL)-6, KC/CXCL1 (murine equivalent to IL-8) as well as gelatinase B (MMP-9) activity were evaluated in the bronchoalveolar lavage (BAL) 4 hours after HNE administration.
Results: In comparison with controls, POL6014 dose-dependently reduced all the parameters measured. The maximum inhibition was observed at the dose of 0.5 mg/kg which reduced the neutrophil influx (41.8 ± 3.8 % *vs.* 4.4 ± 2.2 % increase), Hb content (0.071± 0.011 *vs.* 0.001 ± 0.001 g/dL), IL-6 (209.6 ± 33.8 vs. 39.8 ± 2.9 pg/ml), KC/CXCL1 (693.8 ± 69.6 vs.147.5 ± 12.1 pg/ml) and MMP-9 activity (63.9 ± 9.6 vs. 13.2 ± 1.5 relative zymography densitometry units). Moreover, POL6014 was more potent than ONO-5046 since only a dose of 5 mg/kg ONO-5046 showed a similar level of inhibition as 0.5 mg/kg POL6014.
Conclusion: Inhibition of HNE by a local instillation of POL6014 significantly and efficiently reduced the inflammatory processes of ALI in HNE treated mice. Our observations suggest that POL6014 might provide a new therapeutic approach for the treatment of ALI and potentially other NE-driven lung diseases like CF, COPD and emphysema.

Introduction

Human neutrophil elastase (HNE) is a 29kDa serine protease hydrolysing most components of connective tissue, including elastin (1-2) that imparts structural stability to the lung. HNE has been suggested to participate in the development of emphysema, a main component of chronic obstructive pulmonary disease (COPD), but also to be involved in the secretion of pro-inflammatory mediators and mucus. Although many proteases have been described, HNE may be the most devastating elastolytic enzyme involved in respiratory disorders. Therefore, the blockade of the elastolytic activity with an inhibitor could improve the treatment of emphysema and inflammatory processes of the airways (3-5).
The aim of the study was to evaluate the local effects of POL6014 on HNE-induced pulmonary inflammation in mice and to compare its effects with those of sivelestat (ONO-5046).

Materials and Methods

Animals and HNE administration
Ten-week-old male C57BL/6j mice (CERJ, Le Genest Saint Isle, France) were anesthetized with etomidate *i.p.* and instilled by *i.n.* injection of 25 µL (1 mL/kg) solution of HNE 30 UI/mouse. 15 minutes before HNE administration, mice were treated, with either POL6014 at 5, 0.5, 0.2 and 0.05 mg/kg *i.n*. or sivelestat at 1 and 5 mg/kg *i.n.*
Bronchoalveolar lavage (BAL)
Four hours after HNE instillation, BAL was collected and centrifuged (600*g* for 10 min, 4°C). After lysis of erythrocytes with distilled water followed by osmotic re-equilibration, the cell pellets were suspended in 500 µL of 0.9% NaCl and counted.
Total and differential cell count
Total cell count was evaluated using an hemacytometer chamber and viability was determined by the trypan blue exclusion method. After cytocentrifugation (Cytopro 7620 WESCOR) of 100000 cells, at 700 r.p.m. for 10 min, the cells were stained with May-Grünwald Giemsa. Differential counts on 200 cells were made using standard morphological criteria.
Pro-inflammatory cytokine and MMP-9 levels
The amount of IL-6 and KC in the BAL fluid supernatant was quantified by enzyme-linked immunosorbent assay (ELISA). Using zymography, MMP-9 was detected in BAL through its capacity to degrade gelatin. Enzyme amounts were quantified by measuring the surface and intensity of the lysis bands using densitometric analyser software package (Bioprofile, Vilbert Lourmat, Marne La Vallée, France).
Analysis of hemoglobin
BAL hemoglobin content was determined by a Radiometer ABL125 (Denmark). (6)
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Results

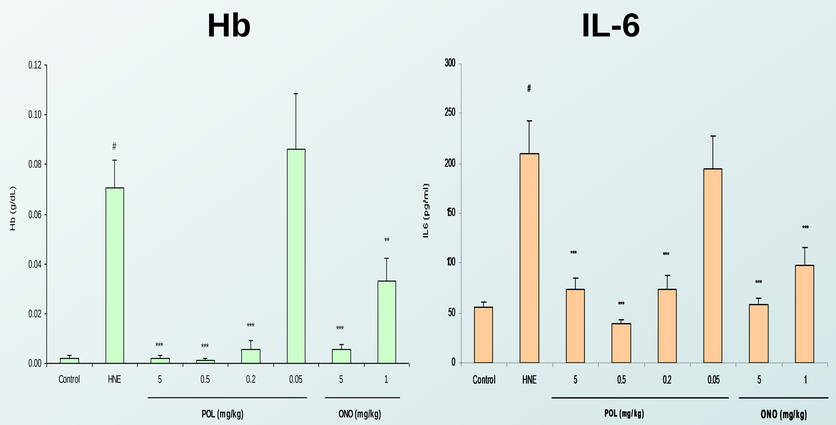

Figure 1: Effect of NE inhibitors, POL6014 and ONO-5046 (ONO) on levels of hemoglobin (Hb), interleukin-6 (IL-6), KC/CXCL1 (KC) and 105KDa MMP-9 gelatinase activity (MMP-9) in bronchoalveolar lavage fluids of mice treated with HNE.
Levels of Hb and mediators were measured in BAL fluids collected at 4 hours after intranasal administration of vehicle (control) or HNE (30 UI). POL6014 (0.05, 0.2, 0.5 and 5 mg/kg) or ONO-5046 (1 and 5 mg/kg) were administered 15 min before HNE.
Results are presented as mean ± sem (n=10-27). #: p<0.001 from mice administered with HNE compared to control. **: p<0.01, ***: p<0.001 compared with mice administered with HNE and treated with NE inhibitors.

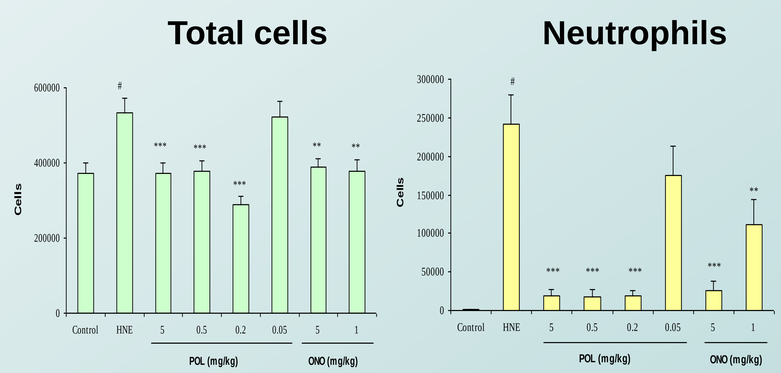

Figure 2: Effect of NE inhibitors, POL6014 and ONO-5046 (ONO) on HNE-induced changes in bronchoalveolar lavage cell content.
Total cells (left) and neutrophils (right) were quantified in BAL fluids collected at 4 hours after intranasal administration of vehicle (control) or HNE (30 UI). POL6014 (0.05, 0.2, 0.5 and 5 mg/kg) or ONO-5046 (1 and 5 mg/kg) were administered 15 min before HNE.
Results are presented as mean ± sem (n=10-27). #: p<0.001 from mice administered with HNE compared to control. **: p<0.01, ***: p<0.001 compared with mice administered with HNE and treated with NE inhibitors.

Conclusions

- **A local instillation of POL6014 significantly and efficiently reduced the inflammatory processes of ALI in mice administered with HNE.**
- **POL6014 might provide a new therapeutic approach for the treatment of ALI and potentially other NE-driven lung diseases like CF, COPD and emphysema.**

References

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ATS, San Diego, May 15-20, 2009