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

Lagente V¹, Guénon I¹, Morel I², Sellier-Kessler O³, Chevalier E³

¹ INSERM U260, Université de Rennes 1, France; ² CHU Pontchaillou, Rennes, France; ³ POLYPHOR Ltd, Allschwil, Switzerland

Abstract

Introduction

Human neutrophil elastase (HNE) is a 29kDa serine protease hydrolyzing 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 (CEUR, 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 U/mouse, 15 minutes before HNE administration, mice were treated, with either POL6014 at 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 (600g 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 different cell count

Total cell count was evaluated using an hemocytometer chamber and viability was determined by the trypan blue exclusion method. After cyt centrifugation (Cytospin 7620 WESCO)® of 10000 cells, at 700 r.p.m. for 10 min, the cells were stained with May-Grünewald Giemsas. 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 activities were quantified by measuring the surface and intensity of the lysis bands using densitometric analyser software package (Biorad, Vilbert Lourmat, Meins La Vallée, France).

Analysis of hemoglobin

BAL hemoglobin content was determined by a Radiometer ABL125 (Denmark).

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 10kDa 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 μL). 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 μL). 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


Figure 2

Figure 1

Figure 2