

PHP-303: A Novel Neutrophil Elastase Inhibitor Reduces Lung Injury In Experimental Acute Respiratory Distress Syndrome

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ABSTRACT

The acute respiratory distress syndrome (ARDS) is characterized by dysregulated inflammation leading to lung injury (ALI). There are no effective pharmacologic therapies for ARDS. We studied the effects of PHP-303 a potent, selective and reversible inhibitor of neutrophil elastase on oleic acid (OA)-induced ARDS in rats. A sham group of rats was studied with five OA-ALI groups treated with placebo (PLCB), three dosages of PHP-303 (3, 10 or 30 mg/kg) or 1 mg/kg Colchicine. Treatments were started 3 days prior to intravenous OA (150 mg/kg)-induced ALI. Four hours after OA treatment, PHP-303 and PLCB groups were compared. PHP-303 reduced histological lung injury area by 88%, lung edema by 50% and improved lung oxygenation by 38%. Lung neutrophil recruitment, assessed by myeloperoxidase (MPO) immunostaining, was significantly increased after OA injury, and this effect was reduced by 31% by PHP-303. PHP-303 also prevented, almost completely, the recruitment of inflammatory cells in bronchoalveolar lavage fluid (BALF), including a 95% reduction of neutrophil recruitment. In BALF, PHP-303 markedly reduced the levels of the pro-inflammatory cytokines; interleukin-6 and MPO, by 71% and 34% respectively. In summary, pretreatment with PHP-303 in a rat ARDS model reduces ALI and respiratory failure with coincident reduction of neutrophil recruitment in lung and BALF and inflammatory cytokines in BALF. This study supports the clinical development of PHP-303 for the prevention of ALI.

INTRODUCTION

- ❖ Model reproducing Acute Respiratory Distress Syndrome (ARDS).
- ❖ Oleic acid (18:1 n-9), an unsaturated long-chain fatty acid, is the most abundant fatty acid in nature and in the human body and it is increased in human ARDS (1).
- ❖ The acute lung damage caused by oleic acid is characterized by important neutrophils accumulation and activation leading to intense inflammation, concordant with the pathophysiology of human ARDS (2).
- ❖ Shared similarities between the oleic acid model of ARDS and coronavirus-induced ARDS are the immune dysregulation with intense inflammation associated with lung neutrophils recruitment as well as cytokine and chemokine elevations (3).
- ❖ PHP-303 (formerly BAY 85-8501) is a small molecule, potent, selective and reversible inhibitor of human neutrophil elastase (4).
- ❖ PHP-303 has been tested in multiple phase I clinical trials (including a 28-day multiple ascending dose study up to 20 mg daily for 28 days) with no severe adverse effects reported.

OBJECTIVES

- ❖ Determine the efficacy of PHP-303 pretreatment to reduce acute lung injury (ALI) in a rat model of OA-induced ARDS.
- ❖ Determine the efficacy of PHP-303 to reduce lung recruitment of inflammatory cells (e.g. neutrophils).

METHODS

108 rats

Terminal analysis:

- ❖ Plethysmography (n=18/group)
- ❖ Arterial blood gas from whole blood (n=18/group)
- ❖ Complete blood count from whole blood (n=18/group)
- ❖ Plasma collection in EDTA vacutainer tube, 6-cc (Cytokines, NETosis, PHP-303 level) (n=18/group)

18 rats in each group were divided into 2 sub-groups for specific lung study purposes:

Sub-group A for lung histological analysis (n=10/group)

- ❖ Left lobe: perfusion-fixed with 10% buffered formalin (lung injury score, leukocyte recruitments, NETosis)
- ❖ Right superior and middle lobes: lung weight and edema assessments
- ❖ Right inferior lobe was fractured into powder by cryogenic grinding process with mortar and pestle at liquid nitrogen temperatures. The fine powder sample was aliquoted into 3 separate sets (n = 10) of micro-centrifuge tubes with > 100 mg of lung tissue in each tube and stored at -80°C for possible cytokine/NETosis marker analysis; PHP-303 concentrations (pH Pharma); and molecular biology analyses.

Sub-group B for bronchoalveolar lavage (n=8/group)

- ❖ Left lung (right lung was ligated): bronchoalveolar lavage procedure with PBS containing protease inhibitor. Three sets of BALF (150 uL) was collected as is for: 1) inflammatory cells counts and 2) quantification of inflammatory cytokines.
- ❖ The remaining BALF was centrifuged and from the supernatant additional sets of aliquots (250 uL) were collected for 1) working out methods and 2) measuring MPO and H3Cit (NETosis markers) and 3) PHP-303 level assessments.

Statistical analyses

- ❖ Bars are mean ± SEM and statistical significance was determined by an ANOVA followed by a Dunnett's multiple comparison test. *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001 vs OA.

RESULTS

Table 1. PHP-303 concentrations ~ 4 hours post final dose

Challenge	Treatment	Plasma PHP-303 (ng/mL)			BALF PHP-303 (ng/mL)		
		n	Mean	SD	n	Mean	SD
Oleic Acid	PHP-303 3 mg/kg	18	264	176	9	993	165
Oleic Acid	PHP-303 10 mg/kg	18	299	113	10	1067	344
Oleic Acid	PHP-303 30 mg/kg	18	288	84	9	1276	264

RESULTS

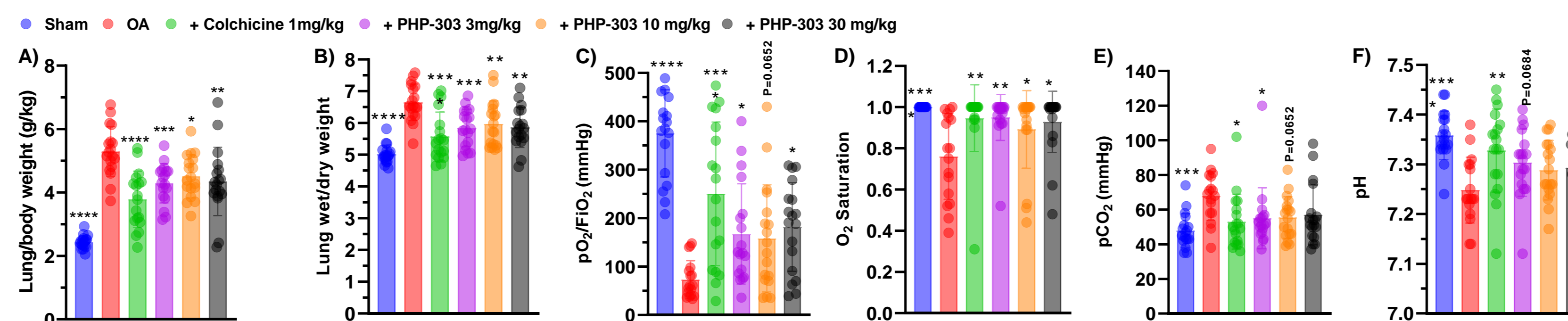


Figure 1. Measurements of lung weight, lung edema and arterial blood gas.

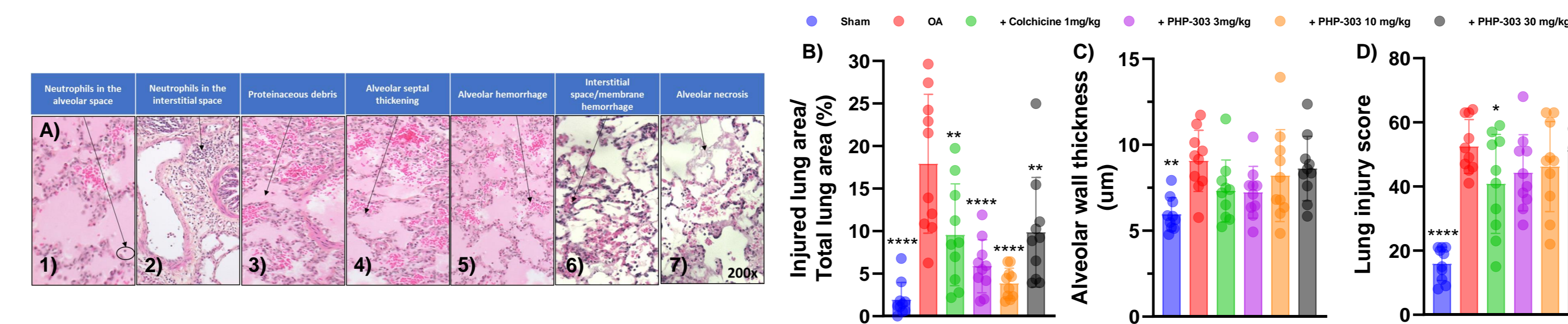


Figure 2. Area of lung injury and injury score. Injury score adapted from the standardized histology score of the American Thoracic Society Documents (5). The histology scores (0, 1 or 2), were given for: 1) neutrophils in the alveolar space, 2) neutrophils in the interstitial space, 3) proteinaceous debris, 4) alveolar septal thickening, 5) alveolar hemorrhage, 6) interstitial space/membrane hemorrhage and 7) alveolar necrosis. For each slide the maximal injury score, corresponding to the sum of the score (score 0 to 2) of the 7 parameters x 5 fields per slide, is 70 points (2x7x5) (6).

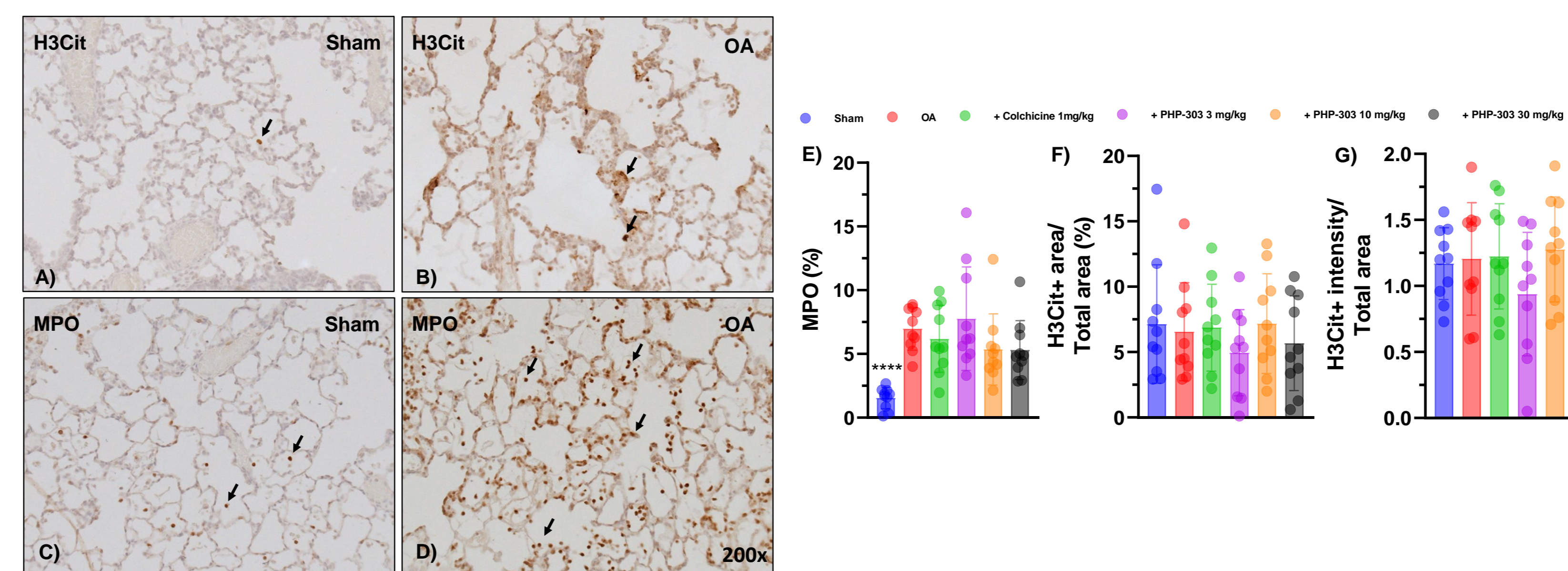


Figure 3. Detection and quantification of neutrophils (MPO) and neutrophils undergoing NETosis (H3Cit) in rat lungs by immunohistochemistry staining.

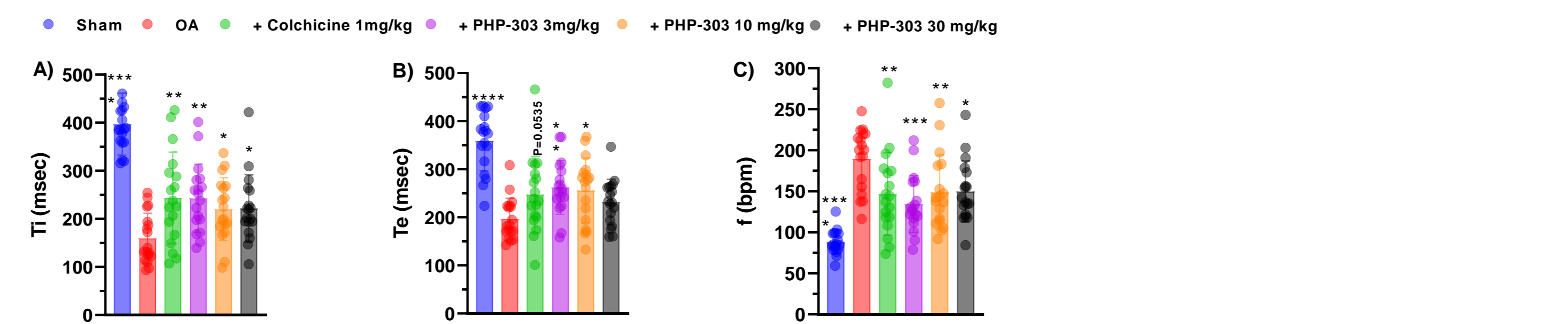


Figure 4. Respiratory parameters by whole body plethysmography 180 min after ALI in conscious unrestrained rats. Inspiratory time (Ti; (A)), expiratory time (Te; (B)) and respiratory rate (f; (C)) were measured.

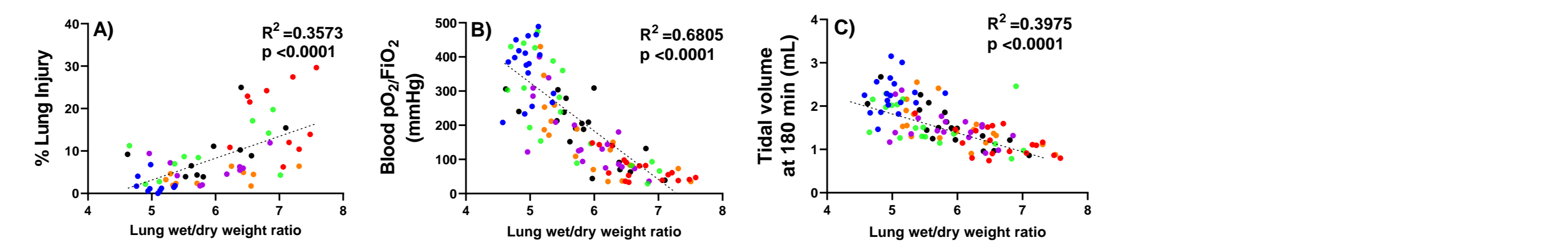


Figure 5. Correlation of severity of ALI with lung edema. Severity of lung edema induced by ALI correlated with histological injury (A) and the severity of respiratory failure (B) causing reduced tidal volume and PO₂/F_iO₂ (C).

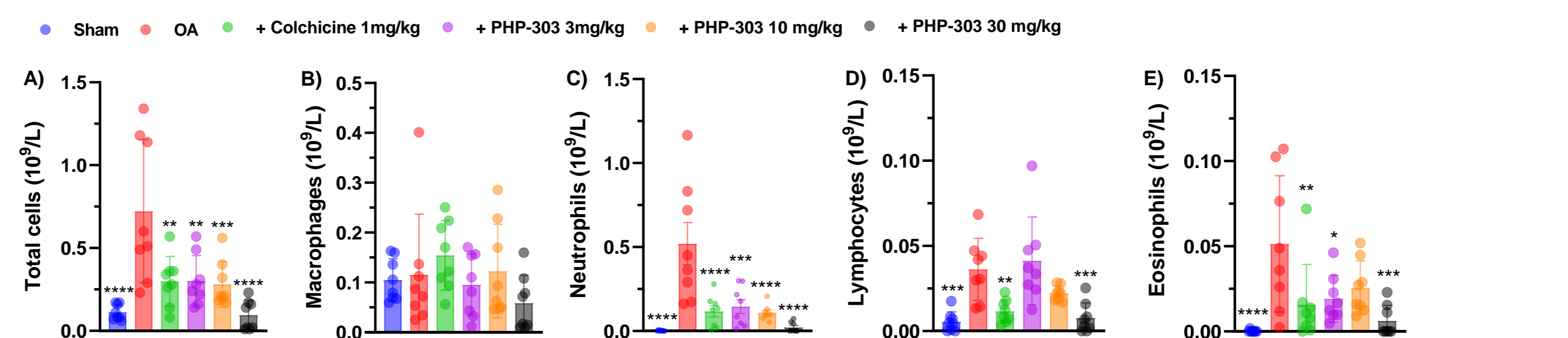


Figure 6. Differential cell counts in bronchoalveolar lavage fluid (BALF).

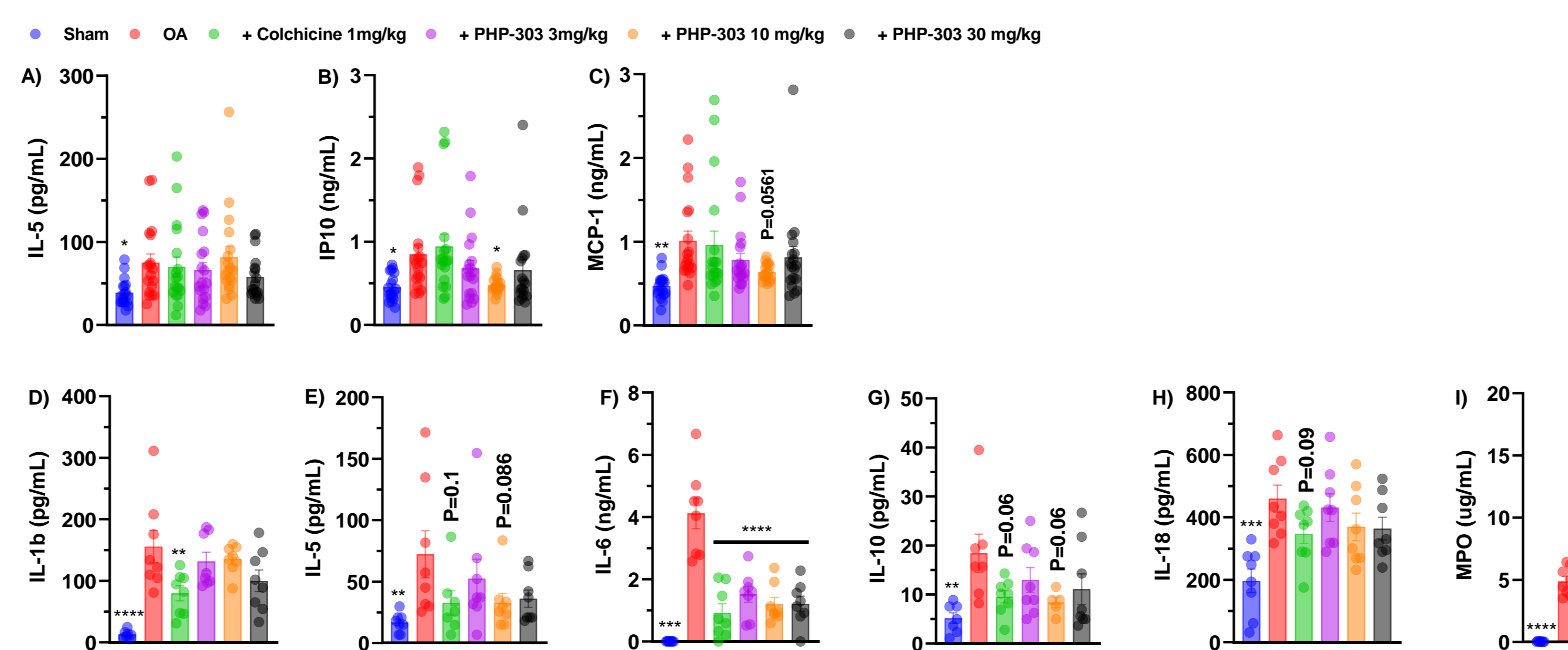


Figure 7. Measurement of cytokines in plasma (A-C) and BALF (D-I). Quantified by ELISA multiplex (EVE-Technologies).

DISCUSSION

- ❖ PHP-303 reduced OA-induced lung injury area by 88%, lung edema by 50% and improved blood oxygenation by 38%.
- ❖ PHP-303 almost completely prevented recruitment of inflammatory cells in BALF, including a 95% reduction for neutrophil.
- ❖ In BALF, PHP-303 markedly reduced the levels of the pro-inflammatory cytokines IL-6 and MPO by 71% and 34% respectively.
- ❖ Low to intermediate doses of PHP-303 appear as effective as the highest dose.
- ❖ This study supports the clinical development of PHP-303 for the prevention of inflammatory ALI.

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- ❖ In conducting research using animals, the investigator(s) adheres to the laws of the United States and regulations of the Department of Agriculture.

CONFLICT OF INTEREST

- ❖ All the authors declare no conflicts of interest.

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