wet:dry weight ratio and lavage fluid total protein were both marginally increased by 10–20 ml/kg V_T compared to non-ventilated controls (NVC). However, raising the V_T to 30 ml/kg did not further enhance these, suggesting that any increases following 10–20 ml/kg were not due to over-stretching the lungs. Only 40 ml/kg induced substantial increases compared to other groups. Both 40 ml/kg and 30 ml/kg upregulated lavage fluid IL-6, while soluble receptor for advanced glycation end-products (sRAGE) tended to be increased with 10–20 ml/kg but not 30 ml/kg (compared to NVC). Again, only 40 ml/kg V_T induced significant upregulation.

Abstract S75 Table 1

	NVC	10 ml/kg	20 ml/kg	30 ml/kg	40 ml/g
End pO ₂ (mm Hg)	—	48.5±3.0	96.3±9.0†	120±3.7†,‡	51.5±9.1‡,§
End BP (mm Hg)	—	55±12	88±8†	86±6†	57±14‡ §
Lung wet:dry ratio	4.90±0.20	5.99±0.42*	6.48±0.85*	5.95±0.48	8.20±0.34* † ‡ §
Total protein (mg/ml)	0.17±0.01	0.91±0.41	0.97±0.30*	0.70±0.40	3.21±0.58* † ‡ §
IL-6 (pg/ml)	16.9±15.9	100±32.0	109±41.5	275±75.3* † ‡	416±108* † ‡ §
sRAGE (ng/ml)	0.43±0.12	34.0±42.0	15.7±22.1	1.95±1.09	84.9±16.7* † ‡ §

N=4–6. Data presented as mean±SD.

*p<0.05 vs NVC;

†p<0.05 vs 10 ml/kg;

‡p<0.05 vs 20 ml/kg;

§p<0.05 vs 30 ml/kg using ANOVA with Bonferroni tests.

Conclusions These data demonstrate that only the highest V_T used (40 ml/kg) induced major changes in physiological and inflammatory markers consistent with development of VILI. Signs of injury/inflammation using V_T 10–20 ml/kg are likely to result not from substantial lung over-stretch but from other factors, particularly epithelial shear stress secondary to alveolar derecruitment and atelectasis. While such V_T may themselves be considered to be “clinically relevant”, whether they induce a “clinically relevant” pathophysiology in healthy mice is questionable.

S76 PROTEINASE 3 ACTIVITY IN SPUTUM FROM ALPHA-1-ANTITRYPSIN DEFICIENT SUBJECTS

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Introduction Tissue destruction in emphysema is widely believed to result from an imbalance between serine proteinases and their inhibitors, the antiproteinases. Previous work has studied the role of neutrophil elastase (NE), but there are few studies of proteinase 3 (PR3). PR3 is released from activated neutrophils concurrently with NE, is more abundant than NE and has fewer airway inhibitors. PR3, unlike NE, is not inhibited by secretory leukoproteinase inhibitor (SLPI). Some studies that have reported “elastase” activity in airway secretions have potentially measured the combined activities of PR3 and NE. Our hypothesis is that PR3 plays a more important role in the tissue damage of emphysema than has previously been considered.

Methods Twenty-eight clinically stable patients with α -1-antitrypsin deficiency (A1ATD) were selected with the PiZZ phenotype and chronic bronchitis. The mean age was 55.8 years and 82% were male. All subjects had full pulmonary function tests and quality of life scores as measured by the St George's Respiratory Questionnaire (SGRQ). Spontaneously produced sputum was collected for microbiology, and the sol-phase was obtained by ultracentrifugation. PR3

and NE activities were measured in all of the samples using specific substrates. The following measurements were taken in a selection of samples; myeloperoxidase (MPO), interleukin (IL)-8, and leukotriene (LT)-B₄. A selection of patients had CT densitometry performed.

Results In the sample of patients studied, PR3 activity was detected in all of the sol-phase sputum samples (mean 323.90 nm, SEM 72.46) whereas NE activity was detected in only 6 of the samples (overall mean 196.42 nm, SEM 100.59). PR3 activity correlated with IL-8 concentration (p=0.004), NE activity (p=0.001) and total pathogenic bacterial load (p=0.001). There was no significant correlation with myeloperoxidase or LTB₄ concentrations. PR3 activity correlated with SGRQ total score (p=0.001) but no correlation was found with lung function parameters or CT densitometry.

Conclusion This pilot study is the first to directly measure PR3 activity in sol-phase sputum. We have shown that PR3 activity can be detected in A1ATD patients, and correlates with the chemo-attractant IL-8, NE activity, pathogenic bacterial load and SGRQ total score. PR3 activity should be assessed when evaluating proteinase-mediated airway damage.

S77 COMPARING THE DEGREE OF INHIBITION OF TNF- α AND IL-6 BY P38 INHIBITORS IN MONOCYTE DERIVED MACROPHAGES (MDMS)

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Objective The activation of p38 MAPK is involved in the increased expression of pro-inflammatory cytokines, such as TNF- α and IL-6, in inflammatory diseases such as chronic obstructive pulmonary disease (COPD). Inhibition of p38 may therefore represent an effective means to combat inflammatory diseases. Inhibitors of p38 such as BIRB796 cause a rapid reduction in the levels of inflammatory cytokines. The aim was to assess the effect of three p38 inhibitors on the lipopolysaccharide (LPS) induced expression of cytokines; TNF- α IL-6 and IL-10 in MDMs.

Methods Peripheral blood mononuclear cells (PBMCs) were isolated from human peripheral blood taken from healthy human donors. Monocytes were subsequently selected through magnetic bead separation. Following the isolation of monocytes, cells were differentiated to MDMs over 12 days in suspension. BIRB796 and two novel p38 inhibitors, PH797804 and PF03715455 were pre-incubated for 1 h before stimulation with 100 ng/ml LPS. Supernatants were collected after 18 h and the levels of TNF- α IL-6 and IL-10 were determined by ELISA. The effects of the compounds were also assessed over 24 h. Supernatants were collected at several time-points allowing the onset and duration of the inhibitory effect to be observed.

Results BIRB796, PHA797804 and PF03715455 caused a substantial inhibition in the release of TNF- α (n=3) and IL-6 (n=3) in LPS-stimulated MDMs. The maximum level of inhibition ranged from 50% to 65%. The addition of the anti-inflammatory cytokine, IL-10, enhanced both the maximum level of inhibition and the potency of each compound. Cytokine expression across 24 h showed a dose dependant inhibition of cytokine release = 2 h (TNF- α) and =4 h (IL-6). The IC₅₀ values in respect to both TNF- α and IL-6 decreased over time.

Conclusion The method described here is an effective means of comparing the effect of p38 inhibitors in MDMs. All three compounds caused a substantial inhibition of, LPS-stimulated, production of both TNF- α and IL-6. The effect of these compounds on IL-10 production requires further investigation. Since a reduction of the anti-inflammatory cytokine IL-10 may counteract the benefits that may be associated with reduced levels inflammatory cytokines due to p38 inhibition in inflammatory diseases.