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Cell pathways in lung inflammation and injury

S73 MACROPHAGES AS VEHICLES FOR DELIVERING CELL THERAPY TO INJURED LUNG

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Introduction Injury to the alveolar epithelium underlies a number of important lung diseases, exemplified by the syndromes of acute lung injury and acute respiratory distress syndrome, which currently have a poor prognosis. Keratinocyte growth factor (KGF) is a mitogen for, and exerts beneficial effects on, type II alveolar epithelial cells. Pre-treatment with KGF is associated with improvements in outcomes in animal models of lung injury, but the use of recombinant KGF as a clinical therapy is limited by its short bioavailability and lack of specificity. We sought to determine whether macrophages could be used as vehicles to deliver KGF therapy to the injured lung.

Methods Macrophages from a murine macrophage cell line (IC-21) were transduced with a lentiviral vector expressing KGF and the reporter gene GFP. Mice were given oropharyngeal (OP) bleomycin to induce lung injury. On days one and three after induction of lung injury, mice were given 3×10^6 KGF-transduced IC-21 cells (or controls) by OP instillation. Mice were sacrificed on day 5, and bronchoalveolar lavage fluid (BALF) was harvested and lungs were processed for histology. For in vivo tracking experiments, IC-21 macrophages were transduced with a lentiviral vector expressing luciferase and mice were imaged longitudinally using real-time bioluminescence imaging.

Results KGF-expression was confirmed in KGF-lentivirus-transduced macrophages, however delivery of these cells was not associated with improvements in measures of alveolar-capillary membrane permeability (BALF albumin) or inflammation (total and differential cell counts) after lung injury. Cells expressing GFP were recovered in BALF, and immunohistochemistry showed groups of cells close to conducting airways. Longitudinal imaging of mice after OP delivery of luciferase-transduced IC-21 cells suggested that cells initially localised to the lungs, but did not persist at 48 h after delivery.

Conclusions KGF-expressing macrophages can be generated using lentiviral vectors, but therapeutic delivery of these cells to the lungs did not improve measured outcomes in the mouse bleomycin lung injury model. Longitudinal imaging suggested that the lack of therapeutic efficacy of KGF-transduced macrophages may be due to their limited survival, and future work should focus on optimising macrophage delivery and survival in vivo.

S74 INCREASED PLASMA LEVELS OF SYNDECAN-1 AND sFLT-1 DURING CARDIOPULMONARY BYPASS SURGERY: ASSOCIATIONS WITH sRAGE

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Introduction and Objectives Endothelial barrier dysfunction contributes to the systemic inflammatory response syndrome (SIRS) for which cardiac surgery necessitating cardiopulmonary bypass (snCPB) is a human model. A growing body of evidence suggests that the receptor for advanced glycation end products (RAGE) is also implicated in the pathogenesis of SIRS. While RAGE activation, in vitro, is known to decrease barrier function of cultured endothelial cells, the role of RAGE in regulating vascular permeability in patients undergoing snCPB is unknown. We hypothesised that plasma levels of markers of endothelial barrier dysfunction, syndecan-1, an endothelial glycocalyx component; and sFlt-1, a soluble form of the vascular endothelial cell growth factor (VEGF) receptor-1, will increase during snCPB and levels relate to patient outcome.

Methods ELISA measurements/western blotting was used to determine syndecan-1 and sFlt-1 levels in pre-, intra- and post-snCPB plasma samples; for comparison, glypican-1, another glycocalyx component and Robo4, an endothelial barrier stabilising protein, were also measured. Clinical indices: age, nature of operation, CPB time, ischaemic time, intensive care unit (ICU)/ hospital length of stay (LOS) were collected from electronic databases.

Results Syndecan-1 was significantly higher during CPB (77.17±39.72 ng/ml) compared with pre-snCPB levels (35.26±25.81 ng/ml, n=14, $p<0.01$). Plasma levels of sFLt-1 were significantly ($p<0.001$) higher during (10 000 pg/ml±3601, n=10) and post-snCPB (4282±3271 pg/ml) compared with preoperative levels (69.68±35.22 pg/ml). Preoperative plasma syndecan-1 correlated positively with ICU LOS ($r^2=0.486$, $p=0.006$); whereas, intraoperative sFlt-1 correlated negatively with ICU LOS ($r^2=0.406$, $p=0.048$). Intraoperative syndecan-1 levels positively associated with ischaemic time ($r^2=0.383$, $p=0.018$) and plasma sRAGE levels ($r^2=389$, $p=0.040$); postoperative syndecan-1 levels correlated with sRAGE ($r^2=0.790$, $p=0.0003$). Glypican-1 and Robo4 were also detected in snCPB plasma samples.

Conclusion Plasma levels of syndecan-1 and sFlt-1 were highest during snCPB. A positive association between preoperative syndecan-1 and ICU LOS is consistent with a relationship between endothelial barrier dysfunction and outcome. By contrast, higher intraoperative sFlt-1 correlating with shorter ICU LOS implied a protective role of sFlt-1. Associations between syndecan-1 and sRAGE suggest a link between RAGE and endothelial barrier dysfunction that merits further investigation; as do the novel findings that glypican-1 and Robo4 were detected in plasma of patients undergoing snCPB.

S75 DO "CLINICALLY RELEVANT" TIDAL VOLUMES REALLY CAUSE VENTILATOR-INDUCED LUNG INJURY IN MICE?

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Background Ventilator-induced lung injury (VILI) caused by excessive lung stretch during mechanical ventilation, is an important determinant of intensive care mortality. In recent years the mouse has increasingly become the pre-clinical model of choice, and studies using mice have identified numerous pathways and mediators all apparently vital during VILI. However, findings have not translated into clinical benefit, and it is conceptually extremely difficult to reconcile this plethora of mediators into a single paradigm. We propose that this confused situation has arisen from a somewhat naïve belief that the wide variety of tidal volumes (V_T) used within such studies all induce over-stretching of the lungs.

Methods Anaesthetised mice were ventilated (3 cm H_2O positive end-expiratory pressure, using air $\pm CO_2$ to regulate pH) with a variety of V_T ranging from "clinically relevant" (10 ml/kg) to "very high" (40 ml/kg) for up to 3 h.

Results Both 10 ml/kg and 40 ml/kg V_T evoked deterioration in arterial pO_2 and mean arterial blood pressure (BP), although intermediate V_T (20–30 ml/kg) did not (see Abstract S75 table 1). Lung

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 chemoattractant 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 IC_{50} 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.