BTS/BLF/BALR young investigators symposium

T1 SIMVASTATIN REDUCES INFLAMMATION AND IMPROVES CLINICAL OUTCOMES IN ALI: RESULTS OF THE HARP STUDY

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Introduction and Objectives There is no effective pharmacological treatment for acute lung injury (ALI). Statins are a potential new therapy as they modify many of the underlying processes important in ALI. The aim of this study was to test whether statins reduce inflammation and improve clinical outcomes in ALI.

Methods We conducted a randomised double-blind placebo-controlled trial in patients with ALI. Patients received 80 mg of simvastatin or placebo for up to 14 days. Measures of pulmonary and non-pulmonary organ function were assessed daily. Pulmonary inflammation was assessed by bronchoalveolar lavage (BAL) cytokines, and systemic inflammation by plasma C-reactive protein (CRP) and cytokines. Data are mean (SD).

Results Sixty patients were recruited. Baseline characteristics, including age, gender and severity of illness scores, were similar in both groups. At day 14, the simvastatin-treated group had significant improvements in oxygenation, respiratory mechanics and overall non-pulmonary organ dysfunction (table 1) with improvement in the cardiovascular ($p = 0.0001$), renal (0.003) and coagulation ($p = 0.04$) SOFA (sequential organ failure assessment) components. At day 14, none of the simvastatin group was receiving vasopressor or inotropes (0% vs 33%; $p<0.05$). There was a trend to a reduction in renal replacement therapy in the statin group at day 14 ($p = 0.09$). Intensive care unit mortality was 30% in both the statin and placebo groups. There was no difference in outcome for patients with sepsis- vs non-sepsis-related ALI. Simvastatin 80 mg was well tolerated, with no increase in adverse events. By day 3 compared with baseline, simvastatin decreased BAL interleukin-8 (IL-8) by 2.5-fold ($p = 0.04$), and IL-6 by 2.9-fold, although this was not significant ($p = 0.07$); these were unchanged in the placebo group. Plasma CRP decreased in both groups but failed to achieve significance in the placebo-treated group (table 1). There was no decrease in plasma cytokines with simvastatin.

Conclusions We have found for the first time that treatment with simvastatin was safe and was associated with improvements in pulmonary and systemic clinical outcomes in ALI. The reduction in pulmonary and systemic inflammatory mediators indicates a potential mechanism for these clinical effects. Phase III trials are warranted to assess the impact of statins in ALI.

Abstract T1 Table 1

	Placebo			Simvastatin		
	Day 0	Day 14	p	Day 0	Day 14	p
Oxygenation index	71.8 (47.5)	73.1 (79.3)	0.95	68.2 (49.8)	23.6 (9.7)	0.01*
Compliance	44.5 (14.1)	53.9 (44.7)	0.31	40 (17.1)	83.4 (49.2)	0.0002*
Lung injury score	2.5 (0.5)	2.2 (1.0)	0.15	2.5 (0.5)	1.5 (0.6)	<0.0001*
SOFA score	10.4 (3.9)	8.8 (5.9)	0.32	10.2 (2.9)	4.2(0.8)	<0.0001*
CRP	209.4 (90.7)	139.4 (111.7)	0.06	206.4 (81.2)	77.61 (72)	0.0003*

CRP, C-reactive protein; SOFA, sequential organ failure assessment.

T2 ENDOTHELIAL PROGENITOR CELLS ARE DYSFUNCTIONAL IN PULMONARY HYPERTENSION

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Background Pulmonary arterial hypertension (PAH) is characterised by disrupted pulmonary vascular homeostasis, muscular hypertrophy of small arteries and, in later disease, areas of disorganised neovascularisation, termed plexiform lesions. Endothelial progenitor cells (EPCs) are involved in vascular regulation and are potential contributors to this process. In addition, EPC function is partly regulated by bone morphogenetic proteins (BMPs), and mutations in the BMP type-II receptor (BMPR-II) are present in >75% of familial and 15% of idiopathic cases of PAH.

Hypothesis EPCs contribute to pulmonary vascular remodelling by dysregulated vascular repair and homeostasis.

Methods and Results Immunohistochemistry was performed to determine expression of progenitor cell markers (CD133 and cKit) and the major EPC homing signals, stromal cell-derived factor-1 (SDF-1) and its chemokine receptor (CXCR4) in explanted lung tissue from patients with PAH. There was significant upregulation of these markers in plexiform lesions only. Levels of putative circulating EPCs (CD34/CD133/VEGFRII+ve), determined by flow cytometry, were increased in idiopathic PAH and BMPR-II-associated PAH, compared with healthy controls. We then isolated late outgrowth progenitor cells from patients with BMPR-II-associated PAH and healthy controls. Cells were stimulated with BMP9 followed by immunoblotting for Smads 1/5/8, the major signalling molecules downstream of BMPR-II. Smad activation was reduced in mutant cells. Quantative PCR confirmed reduced activation of BMP target genes (Id transcription factors) in mutant cells. In functional studies we demonstrated that BMPR-II mutant late outgrowth endothelial progenitors possessed a hyperproliferative phenotype and an impaired ability to form vascular networks.

Summary These findings provide a novel insight into the pathogenesis of plexiform lesions, namely the expansion or recruitment of endogenous EPCs. The major homing signal for EPCs was increased in plexiform lesions in conjunction with an increase in the mobilised fraction of putative progenitors in peripheral blood. Circulating late outgrowth progenitor cells from patients with mutations in BMPR-II have deficient signalling in the BMPR-II pathway and functional differences in their proliferative capacity and ability to form networks in vitro.

Conclusions Taken together these findings provide evidence that progenitor cells are involved in the pathophysiology of vascular remodelling in PAH.

T3 EP4 RECEPTOR-MEDIATED PGE_2 INHIBITION OF AKT PHOSPHORYLATION SENSITISES FIBROTIC LUNG FIBROBLASTS TO FASL-INDUCED APOPTOSIS

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Background We have previously reported that the failure of patients with idiopathic pulmonary fibrosis (IPF) to upregulate cyclooxygenase (COX)-2 and thus the downstream antifibrotic prostanoid prostaglandin (PG) E_2 contributes to disease pathogenesis by increasing fibroblast resistance to apoptosis. To understand the mechanisms involved in this process, we have studied the signalling pathways by which PGE_2 regulates fibroblast apoptosis.

Methods Primary human lung fibroblasts were obtained from IPF surgical biopsies and from control lung obtained at surgical resection. Fibroblast apoptosis was induced by 24 h exposure to Fas ligand (FasL (50 ng/ml)). Cells were additionally treated with either PGE_2, the COX inhibitor indomethacin, 17 phenyl-trinor-PGE_2, butaprost, sulprostone, ONO-A1-329 (E prostanoid (EP) receptor 1–4 agonists, respectively) or ONO-AE3-208 (EP4 antagonist). Apoptosis was detected by annexin V/propidium iodide staining and analysed by flow cytometry. Phosphorylation of the prosurvival protein kinase Akt and XIAP protein levels were determined by western blotting.

Results Fibroblasts from IPF lung (n = 9) are resistant to FasL-induced apoptosis, with levels fivefold lower than in control lung fibroblasts (n = 6). PGE_2 increases FasL-induced apoptosis of fibrotic lung fibroblasts in a concentration-dependent manner. COX inhibition with indomethacin causes apoptosis resistance in control lung fibroblasts. Exposure of fibrotic fibroblasts to the EP4 agonist ONO-A1-329, but not to EP1–3 agonists, increases fibrotic lung fibroblast FasL-induced apoptosis in a concentration-dependent manner and by a similar magnitude to PGE_2. EP4 antagonism inhibits FasL-induced apoptosis in control lung fibroblasts. PGE_2 potently inhibits phosphorylation of Akt in fibrotic lung fibroblasts following FasL exposure. This inhibition is detectable from 30 min through to at least 24 h following addition of FasL. Furthermore, PGE_2 decreases levels of the downstream effector of Akt, X-linked inhibitor of apoptosis protein (XIAP).

Conclusion Reduced PGE_2 in the lungs of patients with IPF promotes fibroblast resistance to apoptosis. PGE_2, when present, increases fibroblast sensitivity to apoptosis via activation of the cAMP-linked EP4 receptor, which in turn inhibits phosphorylation and thus activation of the prosurvival kinase Akt. These results suggest that EP4 receptor agonism offers an attractive therapeutic target in patients with IPF.

T4 VITAMIN D-BINDING PROTEIN AND ALVEOLAR MACROPHAGE ACTIVATION: A MECHANISM FOR THE VITAMIN D AXIS IN LUNG DISEASE

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Background Vitamin D status has been implicated in resistance to both bacterial and viral infections, to which patients with chronic obstructive pulmonary disease (COPD) are susceptible. Most circulating vitamin D is bound to vitamin D-binding protein (VDBP), whose gene contains several variants associated with COPD. VDBP has an indirect role in macrophage activation, a process key to the pathogenesis of airway infections, such that variations in VDBP genotype, level or function might contribute to respiratory phenotype.

Methods 424 clinically characterised PiZZ α-1-antitrypsin-deficient subjects were studied for genetic association with 10 tag single nucleotide polymorphisms (SNPs) covering the VDBP gene, using TaqMan genotyping technologies. Vitamin D levels were measured in serum by tandem mass spectrometry and VDBP levels by ELISA in both serum (n = 60) and sol phase (n = 45) of sputum in a subset of subjects. Alveolar macrophages were obtained from normal lung tissue at resection and the degree of activation in the presence of sol phase from recruited subjects assessed by efferocytosis.

Results Genotyping success rate exceeded 90%, and 9 of 10 SNPs were in Hardy–Weinberg equilibrium. One tag SNP (rs2070741) was associated with bronchiectasis (allele odds ratio (OR) 1.8, p = 0.05) and airway bacterial colonisation (allele OR 3.8, p = 0.04). No genotypic differences in VDBP level were detected.

45% of subjects were vitamin D deficient (<30 ng/ml). Vitamin D exhibited an inverse relationship with its binding protein, such that both related to forced expiratory volume in 1 s (FEV_1), but in opposite directions (vitamin D: r = 0.29, p = 0.02, VDBP: r = −0.32, p = 0.02). Similarly vitamin D tended to be lower in bronchiectatics (mean 17.2 vs 23.0 ng/ml, p = 0.08), and VDBP higher (41.8 vs 25.2 mg/dl, p = 0.09). Efferocytosis of apoptotic neutrophils by alveolar macrophages in response to sol correlated with VDBP level (r = −0.91, p<0.01).

Conclusions VDBP exhibits genetic association and a tendency to higher circulating VDBP levels in bronchiectatics, implying a functional role in disease. VDBP seems to influence efferocytosis, which could be important in persistence of inflammation and airway host defence. Inverse relationships were seen between VDBP and vitamin D, particularly when considering FEV_1, such that associations reported previously for low vitamin D could be due to the effect of concurrent high VDBP.

T5 ESTABLISHING THE PULMONARY TRANSIT TIME OF PRIMED AND UNPRIMED NEUTROPHILS IN MAN

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Studies in humans and animals have suggested that neutrophils transit the pulmonary capillary bed much more slowly than erythrocytes, resulting in physiological pooling of these cells, as occurs in the spleen. Such studies, however, are susceptible to ex vivo neutrophil injury sustained during cell purification and labelling, which results in major alterations in neutrophil rheology and behaviour when re-injected. Neutrophil priming following exposure to inflammatory cytokines has been proposed to be a major factor increasing pulmonary neutrophil retention, predisposing to the development of acute lung injury (ALI)/acute respiratory distress syndrome (ARDS).

We have studied the pulmonary transit of bolus-injected radiolabelled autologous neutrophils using two independent methods: (1) rapid sequence radioisotope imaging of the lungs and (2) arterial outflow detection, in healthy adult human subjects.

Neutrophils isolated and radiolabelled in the presence of autologous plasma (which avoids cell priming and injury) passed through healthy human lungs with a transit time of 14.2 ± 0.3 s (mean ± SEM, n = 8; method 1), at a rate only marginally slower (2.7 ± 1.0 s, n = 6; method 2) than erythrocytes and with <5% first-pass pulmonary retention. Furthermore, 100% of neutrophils primed ex vivo with granulocyte–macrophage colony-stimulating factor (GM-CSF) prior to re-injection were retained on first-pass transit through the lung, with a slow linear washout leaving 35% still retained at 40 min (n = 7). Cells primed ex vivo with platelet-activating factor (PAF), an agent shown to reversibly prime neutrophils in vitro, were initially retained within the lung, as seen with GM-CSF, but thereafter eluted from the lung much faster, so that within 30 min there was no detectable pulmonary retention of neutrophils (n = 6).

These novel findings contradict the previous animal and human studies which suggest that neutrophils are delayed in their transit through the pulmonary capillary bed under physiological conditions, and suggest that the size of the lung granulocyte pool has been overestimated. In addition, we provide the first evidence that neutrophil priming dramatically alters pulmonary transit of these cells in man and that depriming of neutrophils is an in vivo phenomenon. This latter finding suggests that the pulmonary capillary bed may serve the important function of extracting primed cells from the circulation, allowing them to deprime and later releasing them in a quiescent state.

T6 DO DIFFERENCES EXIST BETWEEN NASAL AND BRONCHIAL IN VITRO EPITHELIUM IN CHILDREN WITH AND WITHOUT ASTHMA?

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Introduction It is still unknown whether cultured nasal cells (which are easier to access in children) may be used as a surrogate for bronchial epithelial cells for the study of airway inflammation.

Aim We studied in vitro basal mucociliary differentiation between paediatric nasal (pNEC) and bronchial (pBEC) culture and between those with and without asthma. We looked for differences in epithelial cells when exposed to interleukin-13 (IL-13), the main T helper 2 (Th2) cytokine involved in allergic asthma.

Method Undifferentiated pNECs and pBECs were obtained from brushings (non-bronchoscopic) from children and these cells were differentiated at the air–liquid interface for 28 days. We measured the integrity of tight junctions by measuring transepithelial resistance (TER), goblet cells ($MUC5AC^+$) and ciliated cells (acetylated α-tubulin) in both pNECs and pBECs. We intended to determine if there were differences between nasal and bronchial basal and stimulated measurements and whether these differed between children with and without asthma.

Results Histological cross-sections of the in vitro tissue showed an in vivo-like pseudostratified epithelium in both pNEC and pBEC cultures. Under basal conditions, asthmatic pBEC cultures showed significantly higher goblet and ciliated cells compared with pNECs ($34\pm19.4\%$ vs $14\pm4\%$, $p<0.01$ and $19.3\pm9.8\%$ vs $5.1\pm3.2\%$, $p<0.001$). In non-asthmatic cultures, more ciliated cells were seen in pBECs compared with pNECs ($22.1\pm8.44\%$ vs $8\pm3.1\%$, $p<0.0001$). IL-13 stimulation of non-asthmatic pBECs produced goblet cell hyperplasia to a level similar to the asthmatic basal unstimulated cultures. Asthmatic pNECs exhibited a lower basal goblet cell number which increased with chronic IL-13 exposure. The time profiles for TER showed significant differences between pNECs and pBECs, and stimulation by IL-13 including between sites (nasal vs bronchial) and between those with and without asthma. Most notably, stimulation by IL-13 of asthmatic pBECs showed a significant reduction in TER between days 7 and 14.

Conclusion We observed that differences exist between pNECs and pBECs from children with and without asthma under basal conditions and when they are treated with IL-13. This questions the use of differentiated pNECs for the study of airway inflammation in children with asthma.