Cellular studies in obstructive lung disease

S49 INCREASED SKELETAL MUSCLE-SPECIFIC MICRORNA-1 IN THE BLOOD OF COPD PATIENTS

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Introduction Quadriceps muscle dysfunction is an important prognostic comorbidity in COPD. MicroRNAs (miRs) are small non-coding RNAs that regulate gene expression. Skeletal muscle expresses a number of tissue-specific microRNA including miR-1, which modulates muscle phenotype. MicroRNAs can be secreted from cells and maintained in blood within exosomes. Elevated levels of circulating miR-1 have been demonstrated in a number of human and animal models of muscle disease. We hypothesised that plasma levels of miR-1 would be elevated in COPD patients and would correlate with important physiological parameters.

Methods 103 COPD patients and 25 controls were studied. MiR-1 was quantified in stored plasma samples using q-RT PCR.[1] MiR-16 and miR-122 were quantified as negative controls. Results were normalised to an exogenous spiked-in control.

Results Characteristics as mean (SD); COPD patients: M: 67, F: 36, age=66.47 (8.4), FEV_1 % pred= 43.5 (18.6), 6-minute walk (6MW) = 394 (120). Controls: M: 14, F: 11, age=67 (8.1), FEV_1 % pred=111.2 (13.1), 6MW=613 (83). Plasma miR-1 was significantly elevated in COPD patients, p=0.002. There was no difference in miR-16 and miR-122. MiR-1 was negatively associated with FEV_1 % predicted ($r = -0.3$, $p<0.001$) and with Tlco ($r=-0.3$, $p<0.001$), but it was not possible to distinguish between GOLD stages using ANOVA. However, if patients were sub-divided into early GOLD stage COPD (1 and 2) or late GOLD stage COPD (3 and 4), miR-1 was significantly higher in the latter group (p=0.02). The plasma level of miR-1 was inversely correlated with daily activity measured as locomotion time ($r= -0.25$, $p<0.01$) but miR-1 levels were not associated with any muscle phenotype or with muscle-specific gene expression.

Conclusion Our results show that stable COPD patients have elevated plasma levels of muscle-specific miR-1. The increase in miR-1 may be due to increased muscle degradation or turnover in the COPD patients studied. Our work raises the possibility of using other muscle-specific microRNAs in the future as potential biomarkers of muscle dysfunction in patients with COPD.

REFERENCE

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S50 NITRATIVE STRESS IS INCREASED IN COPD EXACERBATIONS FOLLOWING EXPERIMENTAL RHINOVIRUS INFECTION

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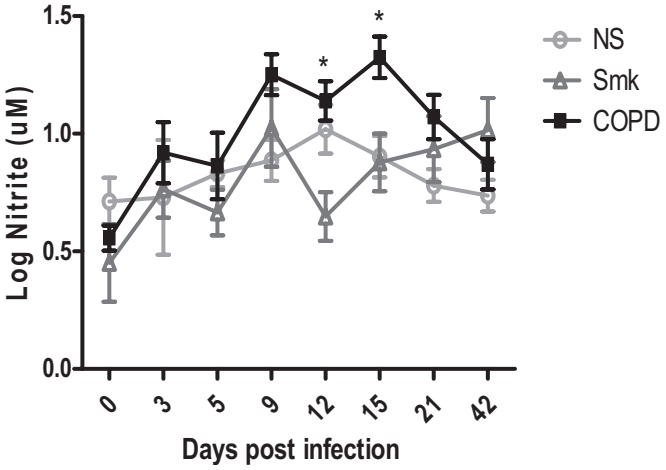
Introduction and Objectives The majority of acute exacerbations of COPD are associated with viral infection and rhinoviruses are the most frequently detected species. Exacerbations represent a major unmet health need and mechanisms are poorly understood. The association between nitrative stress and virus induced exacerbations of COPD are unclear. To investigate this we used an experimental rhinovirus challenge study.

Methods Experimental rhinovirus challenge was performed in COPD (GOLD stage II) subjects (COPD, n=9), and non-obstructed control smokers (Smk, n=10) and non-smokers (NS, n=11). Rhinovirus infection was confirmed with quantitative PCR performed on nasal lavage and sputum samples collected at baseline and days 3, 5, 9, 12, 15, 21 and 42 post virus inoculation. Nitrite concentration was measured in sputum supernatant using the Griess assay, as a marker of total nitrative stress.

Results At baseline the geometric mean (95% CI) nitrite levels were similar between the groups studied (NS 5.13 (3.27 to 8.05); Smk 2.82 (1.37 to 5.80) and COPD 4.17 (3.04 to 5.72); p=0.281). Nitrite levels were significantly higher in COPD subjects on day 15 when compared to both control groups (geometric mean (95% CI) NS 7.94 (7.59 to 8.31); Smk 7.59 (4.41 to 13.04) and COPD 20.98 (13.92 to 31.36); p=0.008). (Abstract S50 figure 1). At every time point sampled there was a significant increase in nitrite concentration from baseline in COPD subjects, but not controls. The area under the curve for nitrite concentration over the time course for NS, Smk and COPD subjects was 18, 43 and 76 respectively ($p<0.05$).

Conclusions Rhinovirus infection is associated with increased nitrative stress in COPD subjects compared to smoking and non-smoking controls. This may play a role in COPD exacerbations.



Abstract S50 Figure 1 Time course of sputum supernatant nitrite levels in COPD subjects and non-obstructed non-smoking (NS) and smoking (Smk) controls. Data presented mean +/-SEM. (* One-way ANOVA $p<0.05$).

S51 REDUCING INTRACELLULAR AGGREGATION AND IMPROVING THE SECRETION OF Z ALPHA-1 ANTITRYPSIN

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The Z variant (Glu342Lys) of α_1-antitrypsin (AT) is one of the serpinopathies; it polymerises and accumulates in the hepatocyte endoplasmic reticulum (ER) resulting in neonatal hepatitis and liver cirrhosis. The secretory defect leaves the lungs vulnerable to elastolysis and early-onset emphysema. Prevention of polymerisation of Z-AT can be achieved in vitro by targeting strand 4a of the AT molecule. Here we evaluate whether an inhibitor of polymerisation; Ac-TTAI-NH_2 (4M) would inhibit Z-AT polymerisation in a cell model. HEK293 cells were transfected with Z-AT (Z-AT cells) or control M-AT (M-AT cells). ELISA demonstrated significantly reduced Z-AT secretion, 242(SEM±63) ng/ml compared to M-AT, 2449±130 ng/ml ($p\leq0.001$), due to retention of Z-AT polymers in inclusion bodies. This was confirmed by electron microscopy demonstrating distension of the rough ER, and ELISA and Immunoblot with a polymer specific antibody (ATZII). 4M significantly reduced the amount of polymers in inclusions in a dose-dependent

manner; no peptide, 2468.5±μg/ml, 5 mg, 2181.7±26.2 ng/ml (p=0.006), 10 mg, 1576±164.7 ng/ml (p=0.001), and 20 and 30 mg completely prevented polymer formation in inclusions (p≤0.001). Unrelated peptides had no effect. Elastase activity of AT in the supernatant from Z-AT cells was significantly reduced compared to M-AT cells; p≤0.001, in keeping with the secretory defect due to retention of Z-AT in inclusion bodies. The elastase activity (and AT concentration) in the supernatant from Z-AT cells was restored by 20 mg 4M, O.D. 405 nm, Z-AT vs Z-AT + 20 μg 4M, 0.129±0.009 vs 0.788±0.054 respectively, (p≤0.001), where a higher O.D. represents higher elastase activity. Functional activity of secreted AT following treatment with 4M was confirmed by its ability to form an SDS-stable complex with elastase as shown by immunoblot. RT-PCR showed that the ER accumulation of Z-AT induced cell stress; NF-κB activation, expression of protein kinase RNA (PKR)-like ER kinase (*PERK*), and IL-6 (100.4±16 pg/ml) and IL-8 (2592.5±575 pg/ml), all of which could be abrogated effectively by 20 mg 4M (IL-6, 45.8±28 pg/ml, p≤0.001 and IL-8, 184.3±29 pg/ml, p=0.014). These findings are the first evidence that inhibitors of Z-AT polymerisation targeting s4A can prevent its cellular accumulation and deleterious effects. Importantly, this strategy was also able to improve plasma concentration of Z-AT.

S52 ASSOCIATION OF MICROTUBULE INSTABILITY WITH DEFECTIVE PHAGOCYTOSIS IN COPD

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Acute exacerbations of COPD are the commonest cause of acute medical admissions in the UK and ~50% are associated with bacterial infection. Alveolar macrophages (AM) normally clear inhaled bacteria but defective phagocytosis may lead to chronic colonisation and increased exacerbations. Monocyte-derived macrophages (MDM), used to model AM, were obtained from COPD, smoking and healthy subjects. MDM phagocytosis of fluorescently-labelled polystyrene beads, *Haemophilus influenzae* (HI) or *Streptococcus pneumoniae* (SP) was measured by fluorimetry. MDM derived from all subjects showed equivalent ability to phagocytose beads, however, COPD and smoker MDM showed significantly reduced phagocytosis of bacteria. Phagocytosis of HI was reduced by 28% and 48% in COPD and smoker MDM respectively, compared to healthy, while SP phagocytosis was reduced by 32% and 52% in COPD and smoker MDM respectively, compared to healthy (Abstract S52 table 1). Having identified defective bacterial phagocytosis in smoker and COPD MDM, the next step was to elucidate the underlying mechanism. Cytoskeletal rearrangement was investigated, with COPD MDM showing significantly reduced phagocytosis of bacteria in comparison to healthy after pre-incubation with nocodazole (microtubule disruptor). Microtubules are involved in membrane trafficking of the phagolysosome and microtubule stability is necessary for effective phagocytosis. Tubulin is acetylated to form stable microtubules and is deacetylated by HDAC6 and Sirt2. COPD MDM showed reduced levels of acetylated tubulin compared to healthy MDM. Pre-incubation with epothilone B (10 nm) a microtubule stabiliser, improved HI phagocytosis in COPD MDM by 20% (p<0.05) and SP phagocytosis in smoker MDM by 40%. Levels of acetylated tubulin increased on exposure to bacteria alone in healthy and smoker MDM but not in COPD MDM. Pre-incubation with epothilone B was associated with significantly increased levels of acetylated tubulin in COPD cells. No significant differences were seen in the expression of HDAC6 or Sirt2 in COPD compared to healthy cells. MDM from smoking and COPD subjects show reduced phagocytosis of common respiratory bacterial pathogens. Acetylation of microtubules appears to be reduced in COPD, whereas, increasing tubulin acetylation is associated with improvements in phagocytosis, which may allow for targeted development of future therapies to treat colonisation and prevent exacerbations of COPD.

Abstract S52 Table 1 Relative fluorescence values (RFU×10^3) for MDM phagocytosis assays at 4 h

	HI	SP
Healthy (n=21)	11.5±0.9 RFU×10^3	8.2±1.4 RFU×10^3
Smoker (n=20)	4.6±0.6 (p<0.001)	3.7±0.6 (p<0.001)
COPD (n=23)	8.4±1 (p<0.01)	5.7±0.7 (p<0.05)

S53 ALARMINS IN BRONCHIOLITIS OBLITERANS SYNDROME AFTER LUNG TRANSPLANTATION

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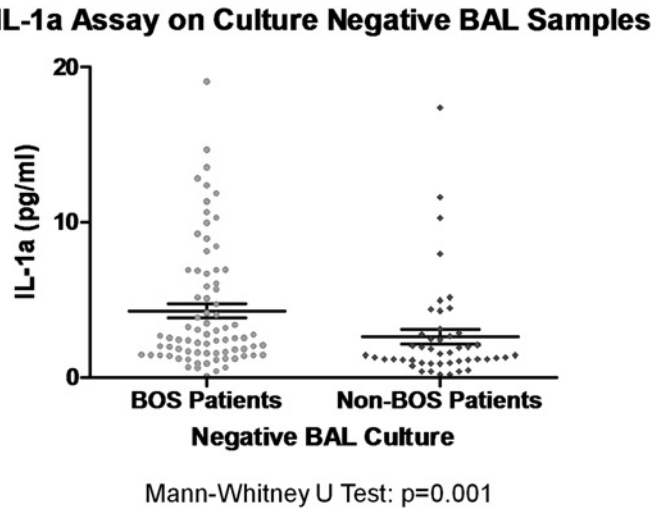
Introduction Survival after lung transplantation is limited to a median of 5-year due to development of bronchiolitis obliterans syndrome (BOS). BOS is the clinical manifestation of chronic allograft dysfunction, characterised by inflammation and fibrosis of small/medium-sized airways leading to airflow obstruction. Numerous insults to the transplanted lungs have been associated with BOS development. Alarmins are cell derived danger signals released from damaged tissue which activate innate and adaptive immune responses. We hypothesised that the release of alarmins into the airway compartment after lung transplantation may contribute to BOS pathogenesis.

Methods A retrospective longitudinal study of 52 lung transplant recipients from 2005 to present was performed (26 recipients developed BOS; 26 remained free from BOS). All recipients had lung function and bronchoalveolar lavage (BAL) performed at 1, 3, 6 and 12 months post transplant. Further samples were taken if the diagnostic criteria for BOS were fulfilled. A total of 214 BAL samples were analysed by ELISA for the alarmins Interleukin-1a (IL-1a) and High Motility Group-Box1 (HMG-B1). Data were analysed using Mann–Whitney tests.

Results Both BOS and non-BOS recipients with culture positive BAL samples had significantly higher concentrations of IL-1a and



Abstract S53 Figure 1 IL-1a assay on culture negative BAL samples.