Early career investigator symposium

T1 ASPIRIN REDUCES PULMONARY INFLAMMATION IN AN INHALED LIPOPOLYSACCHARIDE MODEL OF ACUTE RESPIRATORY DISTRESS SYNDROME (ARDS) IN HEALTHY VOLUNTEERS AND IN A HUMAN EX VIVO LUNG PERFUSION MODEL OF ARDS

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Introduction Platelet activation may play a role in the pathogenesis of ARDS.¹ Animal studies have shown that aspirin therapy reduces pulmonary oedema and development of lung injury.² Our recent observational study has shown patients with ARDS on aspirin had a reduced risk of death.³

Objective To test the hypothesis that aspirin reduces pulmonary inflammation in clinically relevant models of ARDS induced by inhaled lipopolysaccharide (LPS).

Methods Healthy subjects were enrolled in a double-blind, placebo-controlled, allocation concealed study and were randomised to receive aspirin 75 mg or aspirin 1200 mg or placebo (1:1:1) for seven days prior to LPS inhalation. Measurements were performed in bronchoalveolar lavage (BAL) fluid obtained at 6 h after inhaling 50 micrograms of LPS. Parallel experiments were run in an *ex vivo* lung perfusion model (EVLP) using human lungs to determine the effects of aspirin on inflammatory cytokine production and BAL neutrophils in response to intra-bronchial administration of LPS (6 mg).

Results 33 healthy volunteers were enrolled. There was no significant difference between aspirin 75 mg and aspirin 1200 mg. Data for both aspirin groups were combined as per the *a priori* analysis plan. Aspirin pre-treatment reduced LPS-induced BAL neutrophilia (Figure 1a), MMP-9 (33 ng/ml vs 48 ng/ml, $p = 0.03$), the neutrophil-specific protease MMP-8 (3 ng/ml vs 6 ng/ml, $p = 0.03$) and the pro-inflammatory cytokine TNF- α (80 pg/ml vs 106 pg/ml, $p = 0.02$). There was also a non-significant trend towards reduction in a range of inflammatory cytokines (IL-1 β , IL-8 and IL-6).

Pre-treatment with aspirin in the EVLP model also showed a similar reduction in BAL neutrophilia (Figure 1b), along with a trend towards reduction in pro-inflammatory cytokines (IL-8, IL-6, TNF- α , MCP-1).

Conclusion This is the first data to find that aspirin can reduce neutrophilic inflammation in both these models of ARDS. Further clinical studies are planned to assess the role of aspirin in ARDS.

REFERENCES

- 1 Kuebler WM. *J Clin Invest*. 2006 Dec;116(12):3106–8
- 2 Looney MR *et al.* *J Clin Invest*. 2009;119(11): 3450–61
- 3 Boyle A *et al.* *Thorax* 2013;68:Suppl 3 A142–A143

Abstract T1 Figure 1 Aspirin reduces BAL neutrophilia in models of ARDS

T2 VITAMIN D ENHANCES BRONCHIAL EPITHELIAL CELL ANTIOXIDANT RESPONSES AND REDUCES THEIR PRO-INFLAMMATORY CYTOKINE RESPONSE TO STIMULATION BY URBAN PARTICULATE MATTER

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Background Particulate matter (PM) air pollution and vitamin D deficiency are environmental factors associated with asthma exacerbations and severe asthma. PM stimulates cellular inflammatory pathways through oxidative stress and vitamin D has been shown in other organ systems to protect against oxidative stress. We therefore investigated whether vitamin D might protect against PM-induced pro-inflammatory responses.

Methods Primary human bronchial epithelial cells (HBECs) were cultured with ambient PM and/or physiological concentrations of vitamin D. Production of pro-inflammatory cytokines was measured by multiplex bead array, gene transcription by microarray and oxidative stress with appropriate assays.

Results Addition of vitamin D significantly decreased production of IL-6 by PM-stimulated HBECs ($p = 0.011$), however, the reduction was greater in HBECs from healthy ($n = 8$) than from asthmatic ($n = 7$) donors (48.7% vs 28.0% reduction, $p = 0.048$).

Gene transcription microarray identified a subset of pro-inflammatory cytokine genes all down-regulated by vitamin D including *IL6*, *IL24*, *CXCL10* and *CCL20*. Microarray also identified effects of vitamin D on antioxidant genes including *G6PD* (3.1 fold-induction with 1,25(OH)D₃, $p < 0.001$). *G6PD* encodes glucose-6 phosphate dehydrogenase, which is vital for production of reducing equivalents for antioxidant responses.

Vitamin D significantly increased the cellular ratio of reduced to oxidised glutathione (1.6 fold-increase with 25(OH)D₃, $p = 0.042$), enhancing the ability of cellular antioxidant pathways to protectively respond to oxidative stress. Furthermore, addition of vitamin D reduced levels of PM-stimulated 8-isoprostane (19.8% reduction, $p = 0.045$), a marker of oxidative stress damage. Inhibition of *G6PD* reduced the beneficial effect of vitamin D on PM-stimulated HBEC responses.

Conclusion Vitamin D beneficially modulates the response of human bronchial epithelial cells to pathological stimulation by PM, in part through enhancing antioxidant pathway responses. A reduction in PM-stimulated IL-6 is likely important given the association between PM, systemic inflammation and an IL-6 dependent coagulopathy in animal models. Furthermore, many of the vitamin D regulated mediators in the array have profound actions on the adaptive immune system. However, our research has also revealed the novel finding that cells from healthy and asthmatic individuals may respond differently to vitamin D.

T3 THE EFFECT OF ELECTRONIC CIGARETTE EXPOSURE ON INNATE IMMUNE CELLS

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Introduction Are electronic cigarettes (e-cigs) safe? The long-term effects of e-cigs are unknown. E-cigs contain a variety of substances that may be harmful to the lungs. We hypothesised that e-cigs have the potential to cause pulmonary inflammation.

Abstract T3 Table 1 The effects of ECVE exposure on MMP-9 and CXCL8 release from blood neutrophils after 6 hr, and CXCL8 release from alveolar macrophages after 24 hr. Data presented as mean (sd)

Neutrophils				Macrophages	
MMP-9		CXCL8		CXCL8	
0 ECVE	0.003 ECVE	0 ECVE	0.003 ECVE	0 ECVE	0.003 ECVE
		175	348	1647	6000
44782 (25809)	184585 (104617)	(248)	(364)	(2457)	(7602)

We have investigated the effects of e-cigs on human innate immune cells *in vitro*.

Methods Blood neutrophils from six healthy non-smokers were exposed to e-cig vapour extract (ECVE) for 6 hr. MMP-9 and CXCL8 release were measured by ELISA and MMP-9 activity was measured by zymography. p38 MAPK activation was also measured, along with neutrophil shape change and CD11b and CD66b expression by flow cytometry. Finally, we measured CXCL8 release from alveolar macrophages isolated from resected lung tissue from three ex-tobacco smokers exposed to ECVE for 24 hr.

Results Exposure of neutrophils to ECVE increased MMP-9 and CXCL8 release with the maximal effect observed at an optical density (OD) of 0.003 (Table 1). This was observed along with an increase in MMP-9 gelatinase activity and increased p38 MAPK activation.

Furthermore, neutrophil shape change, and dual CD11b and CD66b expression increased in response to ECVE treatment compared to untreated cells.

Following a similar trend, 0.003 (OD) ECVE caused an increase in CXCL8 release from alveolar macrophages.

Discussion We have shown that e-cig exposure causes an inflammatory response from neutrophils and macrophages. The effects discussed here are similar to those caused by tobacco cigarettes. Based on these findings, the use of e-cigs may pose a risk to public health.

T4 PNEUMOCOCCAL CONJUGATE VACCINE REDUCES RATE, DENSITY AND DURATION OF EXPERIMENTAL HUMAN PNEUMOCOCCAL COLONISATION: FIRST HUMAN CHALLENGE TESTING OF A PNEUMOCOCCAL VACCINE

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Objective To determine the effect of the Pneumococcal Conjugate Vaccine (PCV-13) on experimental pneumococcal colonisation compared to Hepatitis A vaccination (control) in healthy participants.

Design Double blind randomised controlled trial.

Setting Clinical Research Unit in the Royal Liverpool University Hospital.

Participants 99 healthy participants aged 18–50 years were randomly assigned to receive PCV-13 (n = 49) or Hepatitis A (n = 50) vaccination according to a randomisation plan in blocks of ten.

Interventions Participants previously vaccinated with PCV or control were inoculated after 4 weeks with 80,000 CFU/100 µl

Abstract T4 Figure 1 Percentage of participants' colonised and non-colonised by 6B pneumococcus assessed using classical microbiology according to vaccination group at any time point

pneumococcal bacteria (6B) 100 µl per naris. Participants were followed up for 21 days to determine pneumococcal colonisation by culture of nasal wash samples.

Main outcome measures The primary outcome measure was the culture of type 6B pneumococcus at either day 2, 7, 14 or 21 following inoculation. Secondary outcome measures included the density and duration of pneumococcal colonisation post inoculation of 6B and the presence of any other naturally acquired pneumococcal strains.

Results The PCV group showed a significantly reduced experimental colonisation rate 5/48 compared to the control group 23/48 (<0.001) [Figure 1]. Both the density and duration of colonisation were reduced in the PCV group compared to the control group following inoculation. The area under the density-time curve (total exposure) was significantly reduced in the PCV compared to control group (mean 8902 vs 267580 p = 0.0179).

Conclusion PCV reduces pneumococcal colonisation rate, density and duration in healthy adults. The Experimental Human Pneumococcal Colonisation (EHPC) model is a safe, effective and efficient method of analysing the efficacy of vaccination on pneumococcal colonisation. We suggest that this novel EHPC model can now be used as a platform for future pneumococcal vaccine testing, using small sample sizes and shorter time scales than community studies in order to reduce time and cost to market. We recommend that carriage rate, density and duration are all measured in these studies.

Trial registration.

EudraCT: 2012-005141-20.

ISRCTN: 45340436.

T5 OPG REGULATES PULMONARY ARTERIAL SMOOTH MUSCLE CELL PROLIFERATION AND THE EXPRESSION OF PAH-ASSOCIATED GENES VIA FAS

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