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

UIH Imran Hamid, JC Conlon, SP Spence, AB Boyle, MF Fitzgerald, MS Shyamsundar, AK Krasnodembskaya, AK Kissenpfennig, RV Verghis, CS Scott, DFM McAuley, CO O'Kane. *Queens University of Belfast, Belfast, UK*

10.1136/thoraxjnl-2014-206260.1

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

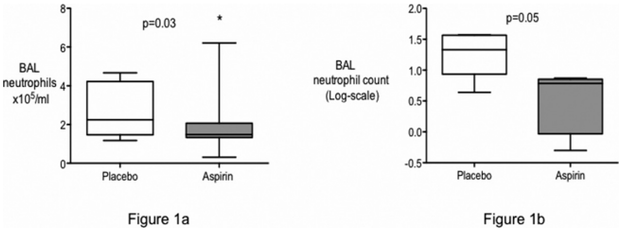

Figure 1a Figure 1b

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

PE Pfeffer, FJ Kelly, CM Hawrylowicz. *King's College London, London, UK*

10.1136/thoraxjnl-2014-206260.2

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

AJ Higham, NJW Rattray, JA Dewhurst, D Singh. *University of Manchester, Manchester, UK*

10.1136/thoraxjnl-2014-206260.3

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.