S76 EXTRAPULMONARY POLYPHARMACY AND CARDIOVASCULAR MEDICATIONS IN COPD

ARC Patel, S Karmali, JJP Goldring, GC Donaldson, JK Quint, JR Hurst, JA Wedzicha. *Academic Unit of Respiratory Medicine, UCL Medical School, Royal Free Campus, London, UK*

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Introduction Cardiovascular co-morbidities requiring pharmacological treatment are common in chronic obstructive pulmonary disease (COPD). However, there are no COPD-specific guidelines and this may lead to suboptimal drug prescribing. We investigated the prevalence and pattern of extrapulmonary medications in COPD and in control subjects.

Methods We retrospectively analysed the recruitment records of 353 patients with COPD and 44 controls (current and ex-smokers with normal lung function) enrolled into the London COPD Cohort. Self-reported co-morbid diagnoses, medication, age, gender and forced expiratory volume in 1 s (FEV_1) % predicted were recorded, and comparisons were made using χ^2 and Mann–Whitney U tests where appropriate.

Results Patients with COPD were prescribed more classes of extrapulmonary medication than controls: median (interquartile range) 3 (1–5) vs 1 (0–3) p = 0.009. 20% of COPD patients were prescribed ⩾5 classes of extrapulmonary medication, fulfilling a definition of polypharmacy, vs 7% of controls (p = 0.037). The use of angiotensin-converting enzyme (ACE) inhibitors, calcium channel antagonists, loop diuretics and nitrates was significantly more common in patients with COPD (see table 1), in keeping with a higher prevalence of cardiovascular disease in this group. Almost half of the patients with COPD reported taking at least one class of blood pressure-lowering medication compared with fewer than a third in the controls. Despite the difference in cardiovascular disease, there was a significantly lower use of β-blockers in patients with COPD compared with controls (4.5% vs 13.6%, p = 0.013). Aspirin and statins were commonly prescribed in both groups (see table 1). Analgesics, antidiabetic, antidepressant and anxiolytic medications were not significantly different in the COPD and control groups.

Conclusions Extrapulmonary polypharmacy is approximately three times more common in patients with COPD than in controls. There are clinically important differences in the pharmacological treatment of cardiovascular disease in COPD compared with controls. In particular, cardioselective β-blockers are significantly underprescribed despite good evidence of their safety and benefit in COPD.

Clear guidelines on the treatment of cardiovascular co-morbidities in COPD may help to reduce morbidity and mortality through the rational use of appropriate medication classes.

Abstract S76 Table 1

	COPD n = 355	Controls n = 44	p Value
Age (mean ± SD)	68.7 ± 8.9	68.8 ± 6.0	0.951
FEV_1 % predicted (mean ± SD)	49.1 ± 19.0	92.6 ± 16.7	<0.001
Median number of medication classes (IQR)	3 (1–5)	1 (0–3)	**0.019**
>5 Extrapulmonary classes of medication	19.8%	6.8%	**0.037**
Any antihypertensive	48.4%	31.8%	**0.037**
Statin	26.4%	22.7%	0.606
Aspirin	24.1%	20.5%	0.594
Calcium channel antagonist	21.8%	9.1%	**0.048**
ACE inhibitor	19.5%	6.8%	**0.039**
Loop diuretic	15.9%	4.5%	**0.045**
Thiazide diuretic	14.7%	6.8%	0.152
Proton pump inhibitor	11.0%	6.8%	0.390
Paracetamol	10.2%	13.6%	0.484
Weak opioid	9.9%	9.1%	0.996
Nitrate	8.2%	0%	**0.048**
Calcium/vitamin D supplement	7.4%	0%	0.063
Angiotensin II receptor antagonist	6.2%	4.5%	0.658
Benzodiazepine	4.8%	2.3%	0.445
β-Blocker	4.5%	13.6%	**0.013**
Bisphosphonate	4.2%	0%	0.163
NSAID	2.8%	2.3%	0.831
Potassium-sparing diuretic	3.7%	0%	0.196

ACE, angiotensin-converting enzyme; COPD, chronic obstructive pulmonary disease; IQR, interquartile range; NSAID, non-steroidal anti-inflammatory drug.

Asthma: basic mechanisms

S77 ELISPOT EVALUATION OF PBMC RESPONSES TO *STAPHYLOCOCCUS AUREUS*

J Perera, B Green, M Arden-Jones, P Howarth. *University of Southampton, Southampton, UK*

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Introduction and Objectives In treatment-resistant severe asthma there is evidence of T cell dysregulation. The basis for this is poorly understood, but bacterial superantigens may contribute, as stimulation of T cells by these molecules is poorly regulated by glucocorticosteroids and superantigens are appreciated to contribute to disease severity in other inflammatory disorders, such as atopic dermatitis and nasal polyposis. To investigate this, peripheral blood mononuclear cells (PBMCs) have been stimulated with *Staphylococcus aureus* enterotoxin B (SEB) along with phytohaemagglutinin (PHA), as a positive control, with ELISPOT evaluation of interferon γ (IFNγ) (T helper 1 (Th1)) and interleukin-4 (IL-4) (Th2), to assess whether there is an altered response to these T cell stimulants in severe asthma.

Methods Overnight ex vivo ELISPOTs were carried out on PBMCs stimulated by SEB (0.004–2.5 μg/ml) and PHA (1.25–10 μg/ml) from 20 subjects with severe asthma, 10 with mild asthma and 10 healthy controls. The number of IL-4- and IFNγ-producing cells was assessed and quantified as spot-forming units (SFU).

Results SEB and PHA both induced a dose-dependent increase in SFU for IL-4 and IFNγ in all subjects, with greater numbers of SFU for IFNγ than for IL-4. There was no difference in response between those with mild asthma and healthy controls, but there was evidence of increased sensitivity to stimulation in those with severe asthma, as compared with both those with mild asthma and healthy controls. This difference was most noticeable for SEB stimulation, with significantly increased numbers of IFNγ SFU in severe asthma compared with mild asthma after stimulation with SEB at 0.004 μg/ml (p<0.002), 0.02 μg/ml (p<0.0001) and 0.01 μg/ml (p<0.004), and for IL-4 SFU at 0.004 μg/ml (p<0.03) and 0.02 μg/ml (p<0.01).

Conclusions These findings indicate that these treatment-resistant subjects with severe asthma managed at step 4/5 of the BTS guidelines have increased peripheral Th1 and Th2 responses to SEB as compared with those with mild asthma and healthy controls, suggestive of an altered T cell receptor (TCR) variable beta chain (V Beta)-restricted T cell stimulatory profile. This is evident despite their current asthma medication and is also consistent with severe asthma having a significant and different systemic component from that in mild disease.

S78 INTERLEUKIN-5 INDUCES GLUCOCORTICOID RESISTANCE IN HUMAN EOSINOPHILS

S Brode, N Farahi, AS Cowburn, JK Juss, AM Condliffe, ER Chilvers. *Respiratory Medicine Division, Department of Medicine, University of Cambridge, Cambridge, UK*

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Background Glucocorticoids (GCs) represent one of the most effective clinical treatments for eosinophil-mediated inflammatory diseases such as asthma and allergic rhinitis. Part of the anti-inflammatory effect of GCs has been attributed to their ability to promote eosinophil apoptosis. Cytokines such as interleukin-5 (IL-5), IL-3, eotaxin-1 and granulocyte–macrophage colony-stimulating factor (GM-CSF) increase the survival of eosinophils by inhibiting their apoptotic cell death, and expression of these cytokines is widely reported in eosinophilic inflammation.
Aim To compare the ability of a novel ''high-affinity'' GC, fluticasone furoate (FF) with fluticasone propionate (FP) and dexamethasone (DEX), to modulate eosinophil apoptosis and their potential to over-ride prosurvival signals from IL-5.
Methods Human eosinophils (>99% pure) and neutrophils (>97% pure) were isolated from healthy volunteers using gradient sedimentation and negative immunobead selection (eosinophils). Eosinophils were pretreated with recombinant IL-5 (0.01–1 ng/ml) for 1 h and cultured with GCs (1×10^{-12}–1×10^{-6} M) for 24 h. Apoptosis was assessed by morphology and flow cytometry (annexin V, propidium iodide).
Results Co-incubation of eosinophils with DEX, FP or FF results in a concentration-dependent increase in apoptosis, with similar efficacy but markedly different potency: DEX $EC_{50} = 2.3\times10^{-8}$ M (95% CI 8.2×10^{-9} to 6.7×10^{-8} M), FP $EC_{50} = 4.3\times10^{-10}$ M (95% CI 9.6×10^{-11} to 1.9×10^{-9} M), FF $EC_{50} = 1.4\times10^{-10}$ M (95% CI 8.2×10^{-11} to 2.3×10^{-10} M). The proapoptotic effect of GCs on eosinophils was inhibited by the GC receptor antagonist RU38486 (mifepristone). Consistent with previously published data, neutrophil apoptosis was inhibited by all three GCs: DEX $IC_{50} = 1.3\times10^{-9}$ M (95% CI 1.1×10^{-10} to 1.6×10^{-8} M), FP $IC_{50} = 4.5\times10^{-9}$ M (95% CI $= 7.9\times10^{-10}$ to 2.6×10^{-8} M), FF $IC_{50} = 1.4\times10^{-10}$ M (95% CI 5.7×10^{-11} to 3.8×10^{-10} M). As expected, preincubation with IL-5 inhibited basal eosinophil apoptosis in a concentration-dependent manner (IL-5 at 0.01 ng/ml = 25.9% (95% CI 13.1% to 40.0%, $p<0.05$), IL-5 at 0.1 ng/ml = 60.8% (95% CI 47.4% to 74.2%, $p<0.001$). In the presence of DEX, FP or FF, preincubation of eosinophils with IL-5 caused a complete concentration-dependent inhibition of the proapoptotic effects of GCs without a shift of the GC concentration curves. This is indicative of a non-competitive antagonism.
Conclusions Our data demonstrate that FP and in particular FF display greater potency in inducing eosinophil apoptosis compared with DEX. However, despite this enhanced potency, physiologically relevant concentrations of IL-5 could fully over-ride the proapoptotic effects of GCs. This may play an important role in acquired GC resistance in inflammatory disease and have a significant impact on clinical management.

S79 αVβ5-MEDIATED TGFβ ACTIVATION BY AIRWAY SMOOTH MUSCLE CELLS IN ASTHMA

[1]AL Tatler, [1]L Jolly, [1]J Porte, [2]C Brightling, [1]AJ Knox, [1]L Pang, [1]G Jenkins. *[1]Respiratory Biomedical Research Unit, University of Nottingham, Nottingham, UK; [2]University of Leicester, Leicester, UK*

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Transforming growth factor β (TGFβ) is implicated in airway remodelling in severe asthma. It is synthesised and released from cells as a latent complex then sequestered in the extracellular matrix. Activation of TGFβ is a critical event in TGFβ bioavailability. Lysophosphatidic acid (LPA) is a bioactive phospholipid that induces contraction and/or the formation of stress fibres in many cell types. It is increased in bronchoalveolar lavage fluid following allergen challenge and may contribute to airway remodelling in asthma. We have previously shown that LPA causes stress fibre formation and TGFβ activation in airway epithelial cells. However, whether LPA can cause TGFβ activation in human airway smooth muscle (HASM) cells to promote airway remodelling is unknown.

We stimulated HASM cells with LPA and used a reporter cell co-culture assay to measure TGFβ activation. The mechanism of activation was investigated using an αVβ5 blocking antibody, the inhibitor of cytoskeletal reorganisation, cytochalasin D, and the β_2 agonist formoterol. The nature of the β5 cytoplasmic domain and cytoskeletal interactions was investigated using CS-1 cells, which express no endogenous β5, transfected with DNA constructs for both full-length β5 and polymorphic β5 that has a 9 bp deletion in its cytoplasmic domain. TGFβ activation in asthmatic HASM cells was compared with non-asthmatic HASM cells.

LPA induced a concentration-dependent increase in TGFβ activity, which was blocked by the αVβ5 antibody and cytochalasin D. Moreover, LPA increased the PAI1 (plasminogen activator inhibitor) gene, which was abrogated by TGFβ and αVβ5 neutralising antibodies, cytochalasin D and formoterol. Furthermore, cells transfected with polymorphic β5 could not activate TGFβ. Co-immunoprecipitation showed that polymorphic β5 does not interact with the cytoskeletal protein talin. Finally we showed that asthmatic HASM cells activate more TGFβ in response to LPA than control cells.

In conclusion, we show that LPA induces cytoskeletal reorganisation and αVβ5-mediated TGFβ activation in HASM cells. Interaction between talin and the β5 subunit is essential for αVβ5-mediated TGFβ activation. Furthermore, HASM cells from patients with asthma activate more TGFβ via αVβ5 than control cells. These data provide a potential mechanism through which uncontrolled airway contraction in asthma may promote airway remodelling through αVβ5 integrin-mediated TGFβ activation.

S80 SCREENING FOR NOVEL ANTI-INFLAMMATORY THERAPIES IN A WHOLE ANIMAL IN VIVO MODEL

AL Robertson, CA Loynes, MK Whyte, SA Renshaw. *University of Sheffield, Sheffield, UK*

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Rationale Diseases of neutrophilic inflammation are common, affect many organ systems including the lung, and respond poorly to current therapies. There is a major unmet need to identify new ways to treat such diseases. Neutrophils are usually removed by macrophages having undergone apoptosis, but during inflammatory diseases survival signals delay this apoptosis, leading to enhanced inflammation.
Methods We have established a tractable model in transgenic zebrafish expressing green fluorescent protein (GFP) in the neutrophil lineage, in which inflammation resolution can be rapidly quantitated in vivo. Sterile physical injury to the tailfin of anaesthetised larvae leads to a reproducible and quantifiable neutrophilic inflammatory response which spontaneously resolves over time. This permits screening of compound libraries to identify compounds which accelerate the resolution of inflammation.
Results Preliminary experiments have demonstrated the practicality of such screens, and have identified several lead compounds that serve as ''proof of principle'', demonstrating the utility of this approach in the identifcation of new immunotherapeutics. From the Spectrum collection (MSdiscovery), 960 compounds were tested, of which 12 were shown to have reproducible effects. These include several known anti-inflammatory agents. Of these, a number have been tested and shown to exhibit dose-dependent