3446
The long-acting β2-agonist formoterol re-establishes the anti-proliferative effect of glucocorticoids in asthmatic airway smooth muscle cells (ASMC)
Michael Roth, Jun Zhong, Chong S'ng, Michael Tamm. Pulmonary Cell Research & Pneumology, University Hospital Basel, Basel, Switzerland

Background: Asthma is characterized by an increased mass of ASMC which show increased proliferation in vitro. As previously reported, glucocorticoids do not have a significant anti-proliferative effect on ASMC of asthmatics while although their anti-inflammatory efficacy is maintained.

Methods: We assessed whether the addition of the long-acting β2-agonist formoterol modifies the anti-proliferative efficacy of glucocorticoids (beclomethasone, budesonide, fluticasone). Primary human ASMC lines from asthmatics and controls were set up from bronchoscopic lung biopsies.

Results: Serum deprived sub-confluent cells were stimulated by 5% serum with or without a single drug or combination for a further 48 and 72 hrs. Serum alone increased cell proliferation in healthy control cells by 65% (48 hrs) and 81% (72 hrs), a significantly higher proliferation response was seen in asthmatic ASMC (82% at 48 hrs; 123% at 72 hrs). In the presence of different glucocorticoids alone serum-induced proliferation was significantly reduced in healthy cells (max. 48%) but not in asthmatic ASMC. Treatment with formoterol alone reduced serum-induced proliferation by max. 24% at any time point, with no differences between asthmatic and healthy ASMC. When formoterol was combined with fluticasone a 78% reduction of ASMC proliferation was achieved.

Conclusions: Our results show that the β2-agonist formoterol re-establishes the anti-proliferative effect of fluticasone in asthmatic airway smooth muscle cells. This synergistic action may exert an anti-remodeling effect on the asthmatic airway supporting the clinical benefit of combination therapy.

3447
A novel target of formoterol, a dual-specificity phosphatase DUSP4 on regulation of corticosteroid budesonide sensitivity
Yoshiki Kobayashi1, Nicolas Mercado1, Anna Miller-Larsson2, Peter Barnes1, Kazuhiro Ino1. 1Airways Disease, NHLI, Imperial College, London, United Kingdom; 2R&D Lund, AstraZeneca, Lund, Sweden

Introduction: We previously reported that formoterol, a long-acting β2-adrenoceptor agonist, activated a serine/threonine protein phosphatase PP2A which is involved in the restoration of corticosteroid (CS) sensitivity by formoterol (Kobayashi et al ERJ 2009;34:583s). We explored other phosphatases and identified dual-specificity phosphatase DUSP4 as a possible novel target of formoterol.

Aims: To confirm that DUSP4 is activated by formoterol and is involved in regulation of CS sensitivity.

Methods: U937 monocytic cells were incubated with IL-2 and IL-4 for 48 h to induce CS insensitivity. CS sensitivity was determined by the CS budesonide ability to inhibit TNFα-induced IL-8 production and to translocate glucocorticoid receptor (GR) from cell cytoplasm to nucleus. Phosphatase activity of immunopurified DUSP4 was measured by fluorescence-based assay. The nuclear/cytoplasmic GR ratio and phosphorylation levels of GR-Ser226 and JNK1 kinase were analysed by western-blotting. In some experiments DUSP4 was knocked down by siRNA.

Results: Formoterol increased DUSP4 activity, which was reduced under IL-2/IL-4 exposure. Knock-down of DUSP4 reduced GR nuclear translocation and CS sensitivity. Knock-down of DUSP4 also abrogated the dephosphorylation of GR-Ser226 and JNK1 by formoterol - the effects involved in GR nuclear translocation and restoration of CS sensitivity.

Conclusions: Formoterol regulates sensitivity to budesonide through activation of DUSP4 which dephosphorylates JNK1 and which in turn leads to dephosphorylation of GR-Ser226 and JNK1 by formoterol - the effects involved in GR nuclear translocation and restoration of CS sensitivity.

3448
Tiotropium reduces established pulmonary inflammation in a 12 weeks cigarette smoke mouse model of COPD
Lutz Wollin, Florian Gantner, Michael Pieper. Respiratory Diseases Research, Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach, Germany

Rationale: Tiotropium bromide (Spiriva®) is the only marketed long acting anticholinergic for the treatment of chronic obstructive pulmonary disease (COPD).
We recently demonstrated that tiotropium reduces established cigarette smoke (CS)-induced lung inflammation in a short term mouse model. 

**Aims:** To investigate the therapeutic anti-inflammatory activity of tiotropium on CS-induced lung inflammation in a chronic 12 weeks mouse model. 

**Methods:** All mice except controls were exposed to CS for 3 weeks (W) to elicit pronounced lung inflammation. Thereafter, 4 groups of mice were either further exposed to CS or room air (RA) with or without tiotropium treatment up to W12. Tiotropium was administered by inhalation (0.1 mg/mL for 5 min) 1 h prior to CS or RA exposure. Cell counts in the bronchoalveolar lavage (BAL) were determined at W3, W9, and W12. 

**Results:** Lung inflammation induced by 3 W of CS exposure was resolved 5 W after termination of smoke exposure regardless of Tiotropium treatment. Continued CS exposure increases lung inflammation to a maximum at W8. Tiotropium treatment of existing inflammation for 5 W completely inhibited the increase in neutrophil number and reduced monocytic inflammation. After 9 W of Tiotropium treatment the number of monocytes was reduced to baseline levels of healthy animals. Neutrophil number was significantly reduced by 58% after 9 W of Tiotropium treatment compared to sham treated CS exposed mice. 

**Conclusion:** The therapeutic anti-inflammatory properties of tiotropium bromide may diminish the original influence of tiotropium in chronic inflammatory airway diseases like COPD. 

**3449**

**Effects of aclidinium on human lung fibroblast activation in vitro**

Javier Mira1, Adela Serrano2, Teresa Perea3, Ricardo Gjuarero3, Amadeur Mira4, Fidel Mira5, Julio Contijo5. 
1CIBERS, Health Institute Carlos III, Valencia, Spain; 2Department of Pharmacology, Faculty of Medicine, University of Valencia, Valencia, Spain; 3Thoracic Surgery Unit, University General Hospital Consortium, Valencia, Spain; 4R&D Centre, Mirablau, Barcelona, Spain; 5Research Unit, University General Hospital Consortium, Valencia, Spain. 

**Introduction:** Muscarinic activation of human lung fibroblasts is associated with pathological remodelling in the airways of patients with asthma and chronic obstructive pulmonary disease (COPD). 

**Aims:** To investigate the in vitro effects of aclidinium bromide, a novel, long-acting muscarinic antagonist, on human lung fibroblast activation. 

**Methods:** Lung fibroblasts, isolated from human bronchus, were pre-incubated with aclidinium (10^-7M-10^-3M), the ERK 1/2 inhibitor PD98059 (10^-6M) or the cAMP analogue dbcAMP (1mM) for 30 min and then exposed to carbachol (10^-5M) for 48 h. Collagen type I and a-smooth muscle actin (αSMA) expression were measured by RT-PCR, Western blot (WB) and immunofluorescence. ERK 1/2 phosphorylation, nuclear factor (NF)κB transcription and cAMP levels by β-2M, Biotrak enzyme immunoassay. Fibroblast proliferation was assessed using a BrdU kit, and fibroblast migration by wound closure assay. 

**Results:** Aclidinium, PD98059 and dbcAMP attenuated carbachol-induced increases in αSMA and collagen type I mRNA and protein levels. Aclidinium and dbcAMP prevented carbachol-induced increases in phospho-ERK 1/2. Carbachol (10^-5M) prevented isoprenaline (1μM)-induced cAMP upregulation, which was completely reversed by aclidinium 10^-7M. Carbachol-dependent increases in lung fibroblast proliferation (2-fold) were reduced by aclidinium 10^-7M (1.1-fold), PD98059 (1.3-fold) and dbcAMP (1.2-fold). Aclidinium 10^-7M, PD98059 and dbcAMP reduced αSMA and NFκB expression respectively. 

**Conclusions:** Aclidinium blocks carbachol-induced lung fibroblast proliferation probably by a direct effect at muscarinic receptors. Aclidinium may alleviate lung fibroblast activation in patients with asthma and COPD. 

**3450**

The pre-clinical pharmacology of the inhaled mucociliary antagonist GSK573719 predicts once-daily clinical dosing 

Dianne I. Lainé1, Mark A. Luttmann2, James J. Foley3, Chris J. Dehaas2, Charles A. GSK573719 demonstrated pM potency (–log pA2=23.9pM) in an acetylcholine (ACh)-induced dose-response contract in conscious guinea pigs, GSK573719 dose-dependently blocked ACh-induced bronchoconstriction in a concentration-dependent manner (1–100nM). Time to 50% restoration of contraction at 10nM was 1600 min (vs 413 min with tiotropium bromide, 10nM). In conscious guinea pigs, GSK573719 dose-dependently blocked ACh-induced bronchoconstriction with long-lasting action; a 2.5μg intratracheal dose elicited >50% bronchoprotection for >24h. 

**Conclusions:** GSK573719 is a potent mucociliary antagonist that demonstrates slow functional reversibility at cloned human M3 receptors and at endogenous mACHr in isolated human bronchus. This profile translates into 24h duration of bronchodiilation in the clinic, suitable as a once-daily treatment for COPD. 

Funded by GSK. *Formerly at GSK.

**3451**

Synergistic bronchoprotective activity of the long-acting beta 2-agonist olodaterol with tiotropium in guinea pigs 

Thierry Bouyoucos1, Paola Casacosta1, Michael Pieper, Andreas Schnapp, Florian Garnett. Respiratory Diseases Research, Boehringer-Ingelheim Pharma GmbH & Co. KG, Biberach an der Riß, Baden Württemberg, Germany. 

Inhaled corticosteroids (ICS) and long-acting β2-agonists (LABAs) fixed dose combinations (FDCs) are widely used for the treatment of asthma while the long-acting muscarinic antagonist (LAMA) tiotropium is a standard therapy of chronic obstructive pulmonary disease (COPD). Additional improvement of lung function might be expected with the use of the triple therapy “ICS + LABA + LAMA”. 

The aim of this study was to assess potential synergistic bronchoprotective activity of olodaterol (LABA) combined with tiotropium (LAMA) and ciclesonide (steroid), against ovalbumin-induced bronchoconstriction in the anaesthetised guinea pig. 

Bronchoconstriction was induced by inhalation of a single dose of ovalbumin (50 μg/kg) and lung resistance was recorded for 10 minutes in anaesthetised guinea pigs. Compounds were given intra-tracheally 2h before OVA challenge. Olodaterol dose-dependently inhibited the ovalbumin-constricted bronchoconstriction with an ED50 of 0.2 μg/kg. Combination of olodaterol with either tiotropium or ciclesonide displayed Montserrat bronchoprotection in a dose-dependent manner with ED50 values 10-fold lower (0.02-0.03 μg/kg) than the ED50 of olodaterol alone (p<0.05). The triple combination led to even better bronchoprotection with an ED50 of 0.003 μg/kg (p<0.05). 

This study shows that olodaterol in combination with tiotropium and ciclesonide elicits potent synergistic bronchoprotective activity in the guinea pig. This effect is significantly higher than the summarized values of the respective monotherapies and supports the concept of the triple therapy. 

**3452**

Mast cell generated cyclooxygenase products protect airway hyperresponsiveness in a model of chronic asthma 

Barbara Fuchs1,2, Lisa Szep1,3, Christine Möller Westerberg2, Maria Eklof2, Linda Swedin3, Sven-Erik Dahlen1, Mikael Adner1, Gunnar Nilsson2,3, Experimental Allergy and Asthma Research, National Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden; 1Department of Medicine, Chalmers University Hospital, Gothenburg, Sweden; 2Department of Medicine, Chalmers University Hospital, Gothenburg, Sweden. 

The cyclooxygenase (COX) product prostanol (PG) E2 is known to act bronchoprotective in asthmatics. Recent preclinical studies identified the COX-terminal microsomal PG synthase-1 to contribute to the formation of protective PGE2. This study investigated whether the release of bronchoprotective COX products is mast cell (MC) dependent. 

Chronic allergic airway inflammation in mice was induced by ovalbumin/dimethyl sulphate (DMSO) exposure and continued during 90 days. COX-inhibition with diclofenac (DFC) was performed. Airway hyperresponsiveness (AHR) was assessed applying forced-oscillation technique in response to methacholine. Contribution of COX-enzymes to AHR was resolved by comparing WC (MC-deficient) to C57Bl/6 wild type (WT) mice (MCs around central airway). 

Central airway resistance, Rc, was pronounced in allergen-challenged WT mice and increased in WT-DFC mice with increased COX-2 expression. COX-inhibition with diclofenac (DFC) was performed. Airway hyperresponsiveness (AHR) was assessed applying forced-oscillation technique in response to methacholine. Contribution of COX-enzymes to AHR was resolved by comparing WC (MC-deficient) to C57Bl/6 wild type (WT) mice (MCs around central airway). 

Central airway resistance, Rc, was pronounced in allergen-challenged WT mice and increased in WT-DFC mice with increased COX-2 expression. COX-inhibition with diclofenac (DFC) was performed. Airway hyperresponsiveness (AHR) was assessed applying forced-oscillation technique in response to methacholine. Contribution of COX-enzymes to AHR was resolved by comparing WC (MC-deficient) to C57Bl/6 wild type (WT) mice (MCs around central airway). 

This study investigated whether the release of bronchoprotective COX products is mast cell (MC) dependent. 

**3453**

Antitussive effect of (-) menthol mediated by nasal trigeminal TRPM8 receptors

Jana Plevkova1, Brendan J. Canning2, Ivan Polacea3, Mariana Brozmannova1, Milos Tata1. 
1Department of Pathophysiology, Jessenius School of Medicine, Comenius University, Martin, Slovakia; 2Slovak Republic; 3Asthma and Allergy Center, Johana Hopkins School of Medicine, Baltimore, United States; 4Department of Medical Biophysics, Jessenius School of Medicine, Martin, Bratislava, Slovak Republic. 

(-) Menthol, main component of the peppermint is exploited in many over-the-
counter drugs for common cold and cough. It has been reported that (-) menthol has antitussive effect in humans and animals. However, there are conflicting evidences against that statement and also case reports of respiratory failure induced by menthol in kids. Based on the recent data about the distribution of the TRPM8 – menthol receptors expressing neurons in the upper airways, and their low proportion within the vagal nerves in lower airways we suppose, that effect of (-) menthol on cough could be mediated by TRPM8 expressed on nasal afferents. To test this hypothesis a segmental airway challenges with (-) menthol were performed in anaesthetized guinea pigs (nose, trachea and lower airways were separately treated by menthol) and under such set up citric acid cough dose response curves (DRC) were constructed according the method described by Canning et al., 2006. The DRC were compared to control provocations. The (-) menthol applied topically to the trachea does not influence cough significantly. (-) menthol vapors applied directly to lower airways via cannula resulted to the tendency to facilitate cough, with visible signs of increased mucus output, loaded breathing and wheezing. The antitussive effect of menthol was observed only in the group treated by nasal (-) menthol vapors, which reduced cough response to CA significantly comparing to control response $p < 0.001$.

Our results correspond with the data describing the TRPM8 distribution in the respiratory tract, and may contribute to explanation of adverse effects induced by menthol inhalation in kids. Supported: VEGA 1/0031/11.