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Change in dilation effect of prednisone at the different stages of COPD
Nataliya Kuzubova1, Elena Lebedeva1, Anatolyi Fedin2.

Corticosteroids are widely used in treatment of COPD. However there is no consensus about their effectiveness at different stages of COPD.

Aim: To evaluate the effect of prednisone (P) on smooth muscle (SM) contraction of bronchi isolated from rats with different stages of COPD.

Methods: Model of COPD was induced in Wistar rats by long-time nitrogen dioxide (NO2) exposure (15 ppm, 1.5 h/day, 60-90 days). Model adequacy was confirmed morphologically and immunohistochemically. The bronchial SM contractility was evaluated at different stages of COPD (15, 30, 60, 90 days NO2 exposure). Bronchi (2-6 generations) with intramural ganglia were isolated and placed in perfusion solution. P (10 μg/ml) was added in perfusion solution. SM contractions were determined at electrical stimulations preganglionic nerves or SM using the electromechanical displacement sensor.

Results: After 15-day exposure NO2 (acute stage) P decreased the amplitude of bronchial SM contraction caused by stimulation of preganglionic nerves to 29.4±2.5% of the initial level (p<0.01), after 30-day exposure – to 14.7±3.8% (p<0.05), at 60- and 90-day exposure effect of P was absent. With SM stimulation the application of P didn’t affect on SM contraction for any duration NO2 exposure. After 90 days in response to SM stimulation prednisone instead of reducing the SM contraction caused its increase up to 111.0±4.9% (p<0.05).

Conclusion: Bronchodilatory effect of P is mediated by neurogenic mechanism. The greatest effect of P manifests in the initial stages of formation of COPD. As the progression of COPD the dilator effect of P is reduced until disappearance (corticosteroid resistance).

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Inhibition of glycogen synthase kinase 3beta reduces GC function in human monocytes via modulation of HDAC2 activity
Ania Ngkel1, Laurie Stevens2, Mike Yeafon1, Iain Kilty3, Ian Adcock1, Paul Kirkham1.

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Rationale: Inhaled corticosteroids (ICS) reduce inflammatory gene expression. This is usually attributed to direct inhibition of inflammatory gene transcription by the glucocorticoid receptor. However, while corticosteroids induce anti-inflammatory gene expression in vitro, this has not been examined in asthmatic subjects taking ICS.

Methods: Bronchial biopsies from atopic asthmatics taking inhaled budesonide (2×200 μg, twice daily for 11 days) or placebo were subjected to gene expression analysis using real-time reverse transcription-polymerase chain reaction. mRNA expression for the corticosteroid-inducible genes: TSC22D3 (GILZ), DUSP1 (MAPK-1), both anti-inflammatory effectors, and FKBP51 (FKBP5), a regulator of glucocorticoid receptor function, was assessed. Cultured pulmonary epithelial and smooth muscle cells were also treated with corticosteroids before gene expression analysis.

Results: Expression of GILZ and FKBP51 were significantly elevated in budesonide-treated subjects compared to placebo. Budesonide also increased GILZ expression in cultured epithelial and smooth muscle cells and immunostaining showed GILZ expression in the airways epithelium and smooth muscle of asthmatic subjects.

Conclusions: Expression of corticosteroid-induced genes, including the anti-inflammatory gene, GILZ, is upregulated in the airways of asthmatic subjects taking medium daily doses of inhaled budesonide. The biological effects of such genes need to be considered when assessing ICS action.

Funded by AstraZeneca.
P1776 Effects of beclomethasone dipropionate and formoterol in reducing oxidative stress induced by cigarette smoke extracts and IL-17

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1Institute of Biomedicine and Molecular Immunology (IBMI Italian National Research Council (CNR), Palermo, Italy; 2Pharma GmbH & Co. KG, Boehringer Ingelheim, Biberach, Germany

Rationale: Oxidative stress is involved in airway inflammatory conditions. Inhaled corticosteroids reduce airway inflammation and the combination with long-acting β2 agonists enhances this effect.

Objective: To investigate whether cigarette smoke extracts (CSE) and interleukin-17A (IL-17A) activate airway epithelial cells to release markers of oxidative/nitrosative stress and to investigate the effect of beclomethasone dipropionate (BDP) and formoterol.

Methods: Human bronchial epithelial cells (16HBE) were stimulated with different concentrations of CSE (from 0 to 10%) to evaluate the expression of IL-17 receptor (IL-17R) and the IL-17-induced ROS release (p<0.05) and their combination synergistically increased the expression of these markers (p<0.001). BDP alone was able to completely restore the baseline values in terms of IL-17R expression (p<0.001) and its combination with formoterol was superior in reducing the ROS and Nitrosylation production (p<0.001).

Conclusions: Cigarette smoke and IL-17A increase the production of oxidative/nitrosative markers in human bronchial epithelial cells, this effect being reduced by BDP either alone or combined with Formoterol.

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P1777 Activated protein phosphatase PP2A by formoterol enhances nuclear translocation of glucocorticoid receptor induced by budesonide

Yoshiki Kobayashi1, Nicolas Mercado1, Anna Miller-Larsson2, Peter Barnes1, Kazuhito Inoue1,2

1Airway Disease, National Heart and Lung Institute, Imperial College, London, United Kingdom; 2R&D Lund, AstraZeneca, Lund, Sweden

Introduction: We have reported that formoterol (FM), a long-acting β2-adrenoceptor agonist, restores corticosteroid (CS) sensitivity by activation of a serine/threonine protein phosphatase PP2A (ERJ 2009;34:583s). However, the molecular mechanisms how FM activates PP2A and restores CS sensitivity have not been elucidated.

Aims: To investigate the mechanism of PP2A activation by FM and the involvement of PP2A in glucocorticoid receptor (GR) nuclear translocation induced by CS budesonide (BUD).

Methods: Phosphatase activity of immunopurified PP2A from U937 monocyte cells was measured by fluorescence-based assay. A549 lung epithelial cells, without functional β2-adrenoceptor, were used as control cells. Direct effect of FM was evaluated using PP2A immunopurified from cell membrane and recombinant PP2A. The effect of BUD, GR-α or β, on GR translocation activity was measured by a reporter gene assay employing luciferase (LUC, 0.195 kbp) as a reporter. FM directly activated PP2A in A549 cells and induced translocation of GR-α and in human epithelial cells, this effect being reduced by BUD alone or BUD plus FM.

Results: FM enhanced GR activity in both A549 and U937 cells and the effects were blocked by a β-adrenoceptor inhibitor (ICI-118551). FM directly activated PP2A and restored CS sensitivity via activation of PP2A. The effect of FM was seen across multiple species including guinea pig, rat, dog, mouse and rabbit.

Conclusion: FM restores CS sensitivity via activation of PP2A and restores CS sensitivity via activation of PP2A. The effect of FM was dose-dependent with an approximate maximal effect at 2.5 μM concentration of FM.

P1778 Effect of fluticasone and formoterol combination therapy on airway remodeling

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Background: Macrophage infiltration, thickening of the basement membrane and increased mass of airway smooth muscle influence asthma airway remodeling. Different cell types contribute to extracellular matrix deposition. Airway smooth muscle cells (ASMCs) not only contract and relax but also proliferate, respond to inflammatory stimuli and produce extracellular matrix. As shown previously, glucocorticoids increase the deposition of extracellular matrix by human ASMC under inflammatory conditions.

Methods: To analyse the effect of the β2-agonist formoterol on glucocorticoid-induced extracellular matrix deposition, primary ASMC cultures were set up from asthmatics and non-asthmatics. Confluent cells were stimulated with 5% serum with or without a single drug or a combination for a further 72 hr with BUD or salmeterol (0.5 µM each). Total extracellular matrix and collagen deposition were monitored by scintillation counts, described earlier.

Results: Compared to non-stimulated cells 5% serum increased matrix and collagen deposition by 35% and 90%, respectively (p<0.001). BUD alone was able to completely reverse this effect and its combination with formoterol was superior in reducing the expression of β1 and β3 integrins and collagen deposition by 36%. In combination, formoterol abolished the stimulating effect of glucocorticoids on matrix and collagen deposition and reduced matrix deposition. This was dose-dependent.

Conclusions: Our data show that β2-agonists combined with glucocorticoids reduce the excessive matrix deposition induced by glucocorticoids alone. Thus, combination therapy may exhibit benefits for asthmatic patients beyond bronchodi-lating and anti-inflammatory effects.

P1779 P1779: A potent and selective β2-adrenoceptor agonist with rapid onset of action

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Background: β2-agonists are standard treatments for asthma and COPD and are variably optimized for a number of key pharmacological properties, such as receptor selectivity, systemic exposure, onset of action and duration of effect. AZD3199 is a novel ultra long acting β2-agonist (uLABA) with improved properties designed to combine 24 hour duration of effect with low systemic exposure and an onset of action similarly rapid to that of formoterol.

Methods: The affinity, potency and efficacy of AZD3199 was measured at human β2-adrenergic receptors. Onset of action was measured as relaxation of contracted guinea pig trachea and human bronchial tissue. Activity at the hERG voltage-dependent potassium channel was determined using electrophysiology. Plasma protein binding was measured in multiple species.

Results: AZD3199 was a potent agonist (6 nM EC50) at the human β2 receptor with an intrinsic activity of 0.8 relative to formoterol. AZD3199 had a rapid onset of action in both guinea pig (22 min) and human (11 min) lung tissue, very similar to formoterol (G-Pg 23 min, human 13 min) and significantly faster than salmeterol (>100 min in both). Similar β2-agonist activity was seen across multiple species including guinea pig, rat, dog, mouse and rabbit. AZD3199 was highly selective (>1500 fold affinity) for the human β2 receptor over human β1 and β3 receptors with no agonism at either receptor. No activity was seen at the hERG channel at concentrations up to 26 µM. High plasma protein binding (>90%) was seen across multiple species offering the potential for reduced systemic exposure.

Conclusion: AZD3199 is a potent and selective uLABA with an onset of action similar to that of formoterol.

P1780 Oxidative stress-induced corticosteroid insensitivity is reversed by formoterol via inhibition of PI3K signalling in peripheral blood mononuclear cells from patients with COPD and severe asthma

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Rationale: COPD patients show a poor response to corticosteroids which has been linked to oxidative stress. Here we show that the long-acting β2-agonist formoterol (FM) reversed corticosteroid insensitivity after oxidative stress via inhibition of phosphoinositide-3-kinase (PI3K) signalling.

Methods: The responsiveness to corticosteroids dexamethasone (DEX, budesonide (BUD) and fluticasone propionate (FP)) was determined in multiple species. DEX at concentrations up to 26 µM reversed DEX insensitivity in PBMCs from patients with COPD and severe asthma.

Results: PBMCs from patients with severe asthma and COPD are less sensitive to DEX compared to healthy volunteers. Although both FM (10-7 M) and salmeterol (SM, 10-7 M) reversed DEX insensitivity in PBMCs of severe asthma, only FM shows this effect in COPD. In U937 cells, exposure to H2O2 decreased BUD and FP sensitivity and increased Akt phosphorylation as a footprint of PI3K activation. FM restored sensitivity to BUD and FP while the effects of SM were weaker and not statistically significant, and FM but not SM, partially inhibited H2O2-induced Akt phosphorylation. H2O2 also decreased SM-induced cAMP production in U937 cells but did not affect the response to FM. The reduction of SM effects by H2O2 was reversed by pre-treatment with a PI3K inhibitor.

Conclusion: These results suggest that FM restored corticosteroid sensitivity via inhibition of PI3K signalling and that a combination of a corticosteroid with FM may be more effective than that with SM in conditions of high oxidative stress, such as in COPD. Funded by AstraZeneca
P1781

Endogenous PGE2 contributes to antigen-induced contractions of guinea pig trachea
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We have previously shown that PGE2 via EP, receptors maintains basal tone of guinea pig trachea (GPT). Our aim was to assess if antagonism of PGE2 also affected antigen-induced contractions.

Isometric responses to administration of ovalbumin (OVA) were recorded in GPT from guinea pigs sensitized to OVA and expressed as % of the maximal contraction to histamine 100 μM. Before challenge with OVA, the EP3 agonist (ONO-8130; 10(nM)) antagonist was given to naive preparations as well as together with different combinations of inhibitors and antagonists of histamine (mepyramine and metiamide), leukotrienes (zileuton) and prostanooids (indomethacin).

As shown previously, inhibition of one or two mediator classes, generally had no significant effects, whereas triple mediator antagonism (antihista-mines+zileuton+indomethacin) abolished the response to OVA challenge. However, EP3 antagonism partly reduced the antigen contraction. Moreover, when the EP3 antagonist was given together with antihistamines and zileuton, the response to OVA was abolished (Table 1).

Table 1. Contraction (% max) to highest OVA dose

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Control</th>
<th>M &amp; M</th>
<th>INDO</th>
<th>ZIL</th>
<th>ZIL + M &amp; M</th>
<th>ONO</th>
<th>ZIL + M &amp; M + INDO</th>
<th>ZIL + M &amp; M + ONO</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>75 ± 5</td>
<td>72 ± 7</td>
<td>84 ± 5</td>
<td>59 ± 4</td>
<td>77 ± 1</td>
<td>83 ± 1</td>
<td>53 ± 3</td>
<td>60 ± 3</td>
</tr>
</tbody>
</table>

*p<0.05 vs control; *p<0.05 vs ZIL + M & M

Effect of indomethacin together with antihistamines and anti-leukotrienes confirms that prostanoids mediate part of the antigen-induced contraction. The new finding is that EP3 antagonist mimics the effect of indomethacin. This suggests that PGE2 is the main prostanoid mediating the antigen-induced contractions of GPT, a preparation that is known to respond similarly to human airways where the effects of PGE2 still await complete delineation.

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Fluticasone propionate inhaled 3 hours after an early allergen reaction partially inhibits the late phase reaction
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Introduction: Inhalation of 800g budesonide (AJRCM 1994;149:1447) or 500g beclometasone (JACT 1993;91:1163) upon resolution of the allergen-induced early phase response inhibits the late phase asthmatic response (LAR) by 39% and 70%, respectively. This has never been tested for the highly lipophilic corticosteroid fluticasone (FP).

Methods: This randomized double-blinded, placebo-controlled, 3-way crossover study (NCT00716963) was conducted in 6, mild asthmatic patients with a history of allergen-induced LAR. Methacholine challenge in the same animal allowed assessment of combined antagonists bronchodilator efficacy. In addition to reducing number of animals used, this approach allowed us to assess the potency of both M3 antagonists and β2-adrenoceptor agonists efficacies in human airways.

Bronchodilators, like β2-agonists and anticholinergics, are a mainstay therapy for asthma and COPD. Recently, the ultra-long acting β2-agonist olodaterol has been found effective for over 24h in asthma and COPD patients. Increased cholinergic tone, common to these patients, may decrease β2-agonist responsiveness. Using a guinea pig model of allergic asthma, we investigated the reversal of allergen-induced AHR by olodaterol, alone and in combination with the long acting anticholinergic tiotropium bromide. Airway responsiveness was assessed as histamine PC100 (provocative concentration increasing pleural pressure by 100%); as baseline (24 h before challenge) and after the early asthmatic reaction (EAR; 5 h after challenge). Phosphate buffered saline (PBS), 1 mM olodaterol and/or 0.1 mM tiotropium (nebulizer concentrations, 3 min) were inhaled at 5.5 h after challenge, followed by PC50 determinations at 6.5 h and at 24 h (after the late asthmatic reaction; LAR). Allergen challenge after the EAR (8.3-fold decrease in PC100), which was unafflicted by PBS. By contrast, olodaterol strongly reversed the AHR after the EAR (11.4-fold increase in PC50). Tiotropium, which slightly reversed the AHR by itself (2.4-fold), synergistically enhanced the effect of olodaterol to 21.0-fold. After the LAR, the PBS-treated animals were still hyperresponsive (2.3-fold decreased PC100). Tiotropium did not affect this AHR, whereas olodaterol, with or without tiotropium, was still protective. In conclusion, endogenous acetylcholine importantly reduces the reversibility of allergen-induced AHR by β2-agonists.

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P1785

A novel dual-agonist challenge model in guinea pigs for assessment of individual and combined muscarinic antagonists and β2 adrenoceptor agonists bronchodilator efficacy
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The guinea pig broncosecretion model has been shown to be predictive for both muscarinic receptor antagonist and β2-adrenoceptor agonist efficacies in humans. Here we describe a novel in vivo model of bronchoconstriction in guinea pigs, stimulated by two different agonists, histamine and methacholine. This dual-agonist challenge model can be used to assess the potency of both M3 agonists and β2-adrenoceptor agonists individually and in combination. This can be extended to investigate compounds with dual activity: Muscarinic receptor antagonists and β2-adrenoceptor agonists (MABA).

Following administration of test compounds three separate bronchoconstrictor challenges were given to each animal. The first challenge was performed using histamine (100 μg/kg intravenously), the second using methacholine (PCLS) and the third was given using propranolol. The β2 receptor was assessed after the challenge with methacholine. The potency was assessed by calculating the response to the compound and the dose-response curve was determined. The significant difference in responses between single- and dual-agonist models was observed for the both M3 agonists and β2-adrenoceptor agonists tested with regards to in vivo potency or duration of action, therefore validating our approach. In addition to reducing number of animals used, this approach allowed us to assess individual and combined potency and duration of action for both M3 agonists and β2-adrenoceptor agonists in single animal. This novel bronchoregulation model provides a robust mechanism for the future testing of MABA compounds.
Neutraligands of CXCL12: Anti-inflammatory activity in an allergic model of asthma

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Introduction: The chemokine CXCL12 plays an important role in inflammation. Our team identified a small molecule neutralizing CXCL12, belonging to the family of chalcone, named C05. C05 inhibits interaction with its receptors, CXCR4 and CXCR7 (Hachet-Haas et al, JBC 283,23189,2008), and the eosinophil infiltration in a mouse model of allergic eosinophilic airway inflammation. We here evaluated the effect of this neutralizing molecule C05 on airway hyperresponsiveness (AHR), inflammation and airway remodelling.

Methods: Nine week-old male Balb/c mice were sensitized to ovalbumin (OVA), prior to an intranasal challenge of OVA or saline on days 18, 20 and 21. OVA (350 µg/ml) vs vehicle was administered i.p. once daily, 2h before each OVA challenge.

Results: OVA induced AHR (whole body plethysmography), eosinophilia, increase in IL-4, IL-5 and mucus secretion in bronchoalveolar lavage fluid, increase in lung collagen, as well as increased IgE and IgG1 in plasma. C05 decreased AHR (44±2%), eosinophilia (48±7%), IL-5 (44±8%), IL-4 (67±10%) in BAL, and lung collagen (101±21%). IgE and IgG1 levels in plasma and IL-4 secretion in BAL were not modified. In addition, C05 did not modify body or spleen weight. Furthermore, C05 did not induce any CXCR4+ cell recruitment in blood as compared to AM03100, a CXCR4 antagonist vs control group (11±1, 30±3 v 4±1%).

Conclusion: The CXCL12 neutraligand therefore appears as a safe and good candidate in this asthma model.

Anti-inflammatory effects of garenoxacin on IL-8 production and ERK1/2 activation induced by lipopolysaccharides in A549 and THP-1 cells

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Objective: The anti-inflammatory properties of macrolides have been applied to the treatment of inflammatory airway diseases. Although the anti-inflammatory properties of macrolides have been reported, no reports are available regarding a newly developed fluoroquinolone, garenoxacin (GRNX). To examine the immunomodulatory effect of GRNX, we examined the transcription and secretion of inflammatory cytokines by human airway epithelial cells and monocytic stimulated with lipopolysaccharide (LPS).

Methods: A human lung epithelial cell line (A549) and a human monocyte cell line (THP-1) were stimulated with LPS and exposed to different concentrations of GRNX. The transcription of interleukin 8 (IL-8) at 3h was measured in cell lysates using real-time PCR. The secretion of IL-8 was measured in the supernatants of the cell cultures at 24h (A549 cells) or 9h (THP-1 cells) using an enzyme-linked immunosorbent assay.

Results: LPS stimulation resulted in a significant increase in the transcription and secretion of IL-8 by A549 and THP-1 cells. Treatment with GRNX significantly inhibited the transcription and secretion of IL-8 by LPS-stimulated cells through inhibition of LPS-induced ERK1/2 phosphorylation.

Conclusions: GRNX has an anti-inflammatory activity through its capacity to alter the secretion of IL-8 from A549 and THP-1 cell lines.

Polymerized type I collagen reverts airway hyperresponsiveness development in a guinea pig asthma model

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Recently, polymerized type I collagen (PolC) has been shown to display anti-inflammatory and anti-fibrotic properties in an asthma model. The effect of PolC in the pathophysiology of asthma is unknown. Our aim was to study the effects of PolC in airway obstruction and responsiveness in a guinea pig model of allergic asthma with remodelled airways. After an initial sensitization protocol, guinea pigs were intermittently exposed to allergen (ovalbumin, OA) applied every 10 days for up to 125 days (asthma model group), receiving a total of 12 OA challenges. The control group received saline solution instead of OA. Some animals from both asthma model and control groups were treated with 0.66 mg/ml PolC aerosols administered every 5 days from day 65 to 120. Airway responsiveness to histamine was evaluated before the first OA challenge and at the sixth and twelfth OA challenges. From the first challenge on, OA induced a transient airway obstruction and a progressive rise in baseline Penh (a broncho-obstruction index), measured by barometric plethysmography, which was not modified by PolC treatments. At the sixth challenge, OA-induced hyperresponsiveness was abolished at twelfth OA challenge by PolC treatment. In a separate guinea pig group euthanized at the sixth OA challenge, airway subepithelial fibrosis (determined by morphometry) and granulocyte infiltration were observed. PolC treatment reduced both, granulocyte and fibrosis observed at the twelfth challenge. Our data suggest that the rise of Penh baseline is not induced by airway fibrosis, and that PolC is a drug which might become a pharmacological tool to reduce fibrosis, inflammation and hyperresponsiveness in asthma.