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P4827

Acute dose- and time-dependent effects of budesonide on airway blood flow in asthma

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Inhaled corticosteroids (ICS) have been shown to decrease airway blood flow (Qaw) via a non-genomic action on airway vascular smooth muscle contraction. We have previously shown a dose-dependent decrease in Qaw with a single inhalation of 360, 720 and 1440 μg budesonide (BUD) in moderate-to-severe asthmatics where Qaw decreased transiently from 12 to 21% after all doses ($p<0.05$ vs baseline and placebo).

Objective: Here, we have investigated the effects of repetitive BUD inhalations on Qaw in moderate-to-severe asthma patients on regular ICS use.

Methods: The 18 subjects enrolled were told to abstain from ICS for at least 36 h before the experiment. Inhalation of 720 μg BUD was given 4 times, separated by 30 min. Qaw, FEV1, blood pressure, heart rate and oxygen saturation were measured before each inhalation and 30, 90, 150, 210 and 270 min after the last dose. A soluble, inert gas-uptake method was used to measure Qaw.

Results: Baseline mean Qaw was 61.33±3.15 μL/min (per mL of lung anatomical dead space) and FEV1 2.3±0.3 L. Numerically, mean Qaw progressively decreased after each BUD inhalation. At 30 min after the last dose, mean Qaw was 28% below baseline ($p<0.05$) and remained 11% below baseline after 270 min. There were no statistically significant changes in FEV1, FEF 25-75%, PEF, oxygen saturation and mean blood pressure.

Conclusions: In moderate-to-severe asthma patients on regular ICS use, repeated inhalations of high BUD dose have a cumulative acute vasoconstrictive effect in the airway suggesting an acute non-genomic action that increases vasomotor tone. This effect could decrease airway obstruction and the vascular clearance of concomitantly inhaled bronchodilators from the airway.

P4828

Budesonide reverses IL-13–induced airway hyper-responsiveness but has little effect on β2 agonist response in human small airways

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IL-13 modulates airway smooth muscle sensitivity to contractile stimulus. Steroids and β2 adrenoceptor (AR) agonists decrease inflammation and inhibit airway hyper-responsiveness (AHR) in asthma. We postulate that steroids decrease AHR after IL-13 stimulation, and IL-13 alters bronchodilation of small airways.

Precision cut lung slices (PCLS) from disease-free donors were incubated with 100 ng/mL IL-13 (18 h) and examined for carbachol (Cch)-induced bronchoconstriction, and formoterol- (Form) or forskolin (Fsk)-induced bronchodilation. To assess the effect of steroids, slices were preincubated with budesonide (Bud) for 1 h prior to IL-13. Data shown are mean % change of baseline luminal area ± sem. IL-13 significantly increased bronchoconstriction to a maximal effective concentration (100 μM) of Cch (Control (C): -80±4, IL-13: -89±3, p=0.02) and decreased the bronchodilation to 0.3 nM Form (C: 54±8, IL-13: 20±4, p<0.01). 10 nM Bud significantly decreased the AHR to Cch following IL-13 (IL-13: -89±3, IL-13/Bud: -80±3, p=0.01), but had little effect on IL-13-induced impairment of the Form response (IL-13: 20±4, IL-13/Bud: 28±7, p=0.2). In contrast, bronchodilation to 100 μM Fsk was rescued by Bud (IL-13: 42±8, IL-13/Bud: 82±8, p<0.01; C: 76±5, IL-13/Bud: 82±8, p=0.6).

These data suggest that pretreatment with budesonide completely prevents the effects of IL-13 on both airway contractility and adenylyl cyclase-mediated bronchodilation but does not prevent the IL-13-induced impairment of β2AR agonist-mediated bronchodilation. Further studies will define the underlying mechanisms by which IL-13 attenuates β2AR-mediated bronchodilation.

P4829

Dose escalation study in healthy male subjects to investigate safety, tolerability and systemic exposure of orally inhaled single-doses of AP301

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Pulmonary edema is a major complication of Acute Lung Injury (ALI) and Acute Respiratory Distress Syndrome (ARDS) which is associated with higher mortality. AP301 is a synthetic peptide whose structure is based on the lectin-like domain of human Tumour Necrosis Factor alpha. The water soluble peptide can be administered into the lung by oral inhalation. AP301 was designed to activate the pulmonary epithelial sodium channel (ENaC) in type II alveolar cells to accelerate alveolar liquid clearance.

We report the early clinical development of AP301: In the Phase I monocentric FiM trial "Dose escalation study in healthy male subjects to investigate safety, tolerability and systemic exposure of orally inhaled single-doses of AP301" the safety, tolerability and pharmacokinetic profile of AP301 was evaluated in a double-blind, randomized, placebo-controlled, parallel group study that started in April 2011 and was completed in October 2011 at the General Hospital in Vienna. 48 healthy young males received escalating doses of aerosolized AP301 in 6 dose groups between 0.07 mg/kg to 2 mg/kg AP301 per inhalation.

Lung function parameters like FEV1 or PEF were not affected by AP301. Exhaled nitric oxide did not increase. Physical examinations showed no inhalation-related clinical signs or symptoms of paradoxical bronchospasm. No local reactions in the mouth like severe xerostomia or burning sensation were described. Vital signs, ECG and safety laboratory parameters showed no pathological findings. AP301 did not accumulate in plasma.

This phase I trial demonstrated that orally inhaled AP301 was safe and well-tolerated by all study subjects.

P4830

ENaC-activating effect of AP301 in type II alveolar cells isolated from dog, pig and rat lungs

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The synthetic peptide, AP301, mimics the TIP, or lectin-like, domain of human TNF-α (Lucas, R. et al Science 1994; 263:814-817). TNF-α and AP301 activate sodium uptake through the amiloride-sensitive sodium channel (ENaC) in alveolar epithelial cells (AEC), their oedema-reducing capacity having been demonstrated in various studies in rodents. AP301 has also been shown to improve alveolar liquid clearance and lung function in a porcine model of ALI.

Ventilation strategies excepted, no specific therapy presently exists for treatment of pulmonary permeability oedema, ALI or ARDS. AP301, currently undergoing clinical trials, is being developed as a therapy for these conditions.

For pre-clinical regulatory assessment, dog, pig and rat are standard models; accordingly, pre-clinical toxicology studies have been conducted with AP301 in dogs and rats. Hitherto, no studies have assessed the effect of AP301 on primary canine or porcine type II AEC. This study describes the pharmacodynamic effect of AP301 on type II AEC isolated from dog, pig and rat lungs. In whole cell patch clamp experiments with dog type II AEC, AP301 increased the amiloride-sensitive Na current from a control value of 6.1±2.4 pA to 51.8±1.9 pA; in pig type II AEC, an increase from 23.63±1.32 to 173.23±7.44 pA was observed, and in rat AEC, from 10.7±3.8 pA to 66.2±5.3 pA.

These results show that AP301 activates ENaC in type II AEC from dog, pig and rat. To our knowledge, this is the first cell-based analysis of the oedema-clearing effect of AP301 observed in the porcine model of pulmonary oedema. Furthermore, the results validate the dog and pig models in pre-clinical assessment of AP301.

P4831

MEMP1972A, an anti-M1 prime monoclonal antibody, reduces serum IgE in healthy and allergic rhinitis subjects

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Background: MEMP1972A, a humanized monoclonal antibody specific for the M1 prime epitope of membrane IgE, depletes M1 prime-expressing IgE-switched B cells, IgE memory B cells and IgE plasmablasts. MEMP1972A is in development for the treatment for allergic asthma.

Aim: To evaluate the safety, pharmacokinetics (PK) and pharmacodynamics (PD) of MEMP1972A in healthy and allergic rhinitis (AR) subjects.

Methods: Two Phase I, randomized, controlled trials investigated the safety, tolerability, PK and PD of MEMP1972A in (1) healthy adults (n=31 MEMP1972A, n=14 placebo [PB]) and (2) AR subjects (n=24 MEMP1972A, n=12 PB [NCT01160861]). In healthy adults, MEMP1972A was given as single escalating doses of 0.003–5 mg/kg intravenous (IV) or 3 mg/kg subcutaneous (SC). In AR subjects, monthly doses were given for 3 months at 1.5 and 5 mg/kg IV and 3 mg/kg SC.

Results: MEMP1972A was well tolerated, and the exposure of serum MEMP1972A was dose proportional. Following IV administration, the mean terminal half-life of MEMP1972A was 20–21 days and mean clearance was 2.2–2.7 mL/day/kg. MEMP1972A administration led to a dose dependent reduction in serum IgE in both studies. In healthy adults, a single dose of MEMP1972A at 3 and 5 mg/kg IV significantly reduced serum IgE by ∼25% relative to baseline at Day 85, with no significant reductions observed in the PB, lower IV or 3 mg/kg SC cohorts. Monthly doses of MEMP1972A at 5 mg/kg IV or 3 mg/kg SC in AR subjects reduced serum IgE by ∼25% relative to baseline at Day 85. Serum IgE reductions were sustained for 6 months.

Conclusion: MEMP1972A was well tolerated and reduced serum IgE in adults with or without allergic disease.

P4832

IgE-induced pro-inflammatory extracellular matrix composition in airway smooth muscle cells is prevented by omalizumab

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Background: Increased extracellular matrix deposition, and more important its pro-inflammatory composition is a hallmark of airway remodelling in allergic asthma.

Objective: a) To investigate the remodelling potency of antibody activated IgE in human airway smooth muscle cells of asthma and control patients. b) the role of the two IgE receptors on IgE-induced airway remodelling, and c) the potential of the anti-IgE antibody drug omalizumab to prevent IgE-induced extracellular matrix remodelling.

Methods: Isolated primary human airway smooth muscle cells of patients with asthma (n=8) and controls (n=8) were pre-incubated with Omalizumab for 30 min and then stimulated with antibody activated human IgE, and or TGF-β1 for up to 72 hrs. The content of specific collagens in the extracellular matrix was determined by ELISA. IgE receptors were down-regulated by silencing RNA.

Results: IgE and TGF-β1 increased the deposition of collagen-I, -III, and fibronectin, but not of collagen-IV and –VII in asthmatic and non-asthmatic cells. When combined, the stimuli had a synergistic effect. Pre-incubation (30 min) with omalizumab dose-dependently prevented IgE-induced collagen deposition, but had no effect on TGF-β1. IgE stimulation of collagen-I involved IgE receptor-I and p38 MAP kinase. IgE induced collagen-III and fibronectin deposition required IgE receptors-I and –II, as well as Erk1/2 and p38 mitogen activated protein kinases.

Conclusion: IgE is a potent inducer of pro-inflammatory extracellular matrix components in the human airway wall and its effect can be prevented by Omalizumab. Thus the anti-IgE antibody drug may reduce airway remodelling in long term therapy.

P4833

The effects of extrafine beclomethasone/formoterol on hyperinflation and airway geometry in COPD patients

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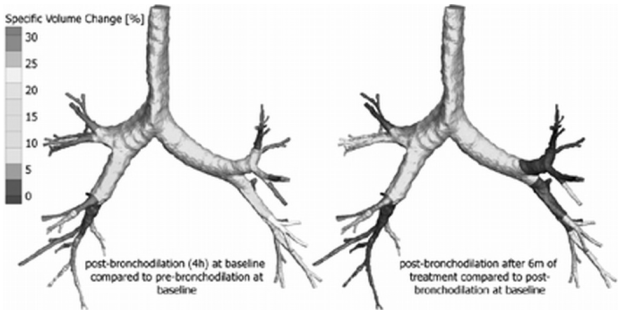
Aim of this study was to assess the effects of extrafine beclomethasone/formoterol treatment on lung hyperinflation and airway geometry in COPD. Data of lobar volume (%pred) and specific lobar airway volumes (siVaw) at FRC and TLC were obtained with functional imaging in 25 COPD patients (GOLD II 14, III 7, IV 4) pre- and 4h post-bronchodilator (post BD) both at baseline and after 6 months of treatment. A post BD drop was observed for both functional residual capacity (FRC) and total lung capacity (TLC) lobar volumes at baseline (FRC: -10%, $p<0.01$; TLC: -2%, $p<0.01$) and after 6 months (FRC: -12%, $p<0.01$; TLC: -2%, $p<0.01$) as index of reduced hyperinflation. siVaw did increase 4 hours after administration at both time points (+10%, $p<0.01$, and +8%,$p<0.01$).



The 4h post-BD drop in hyperinflation at FRC was positively associated with the degree of hyperinflation ($r=0.4$, $p<0.01$). A drop in pre-bronchodilation hyperinflation was also observed at TLC after 6 months of treatment (-1%, $p<0.01$). Extrafine beclomethasone/formoterol decreased hyperinflation and increased airway volume in COPD patients 4h after bronchodilator. Moreover the chronic treatment over 6 months decreased also the pre-bronchodilator hyperinflation at TLC, indicating a progressive reduction of air trapping with treatment.

P4834

Formoterol reduces asthmatic airway smooth muscle cell proliferation through p27 (Kip) which is supported by steroids

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Background: Airway remodelling in asthma is partly due to increased airway smooth muscle (ASM) mass. We and others reported earlier that steroids alone do not significantly reduce proliferation of ASM cells isolated from asthma patients.
Objective: Here, we investigated the anti-proliferative signalling pathway of formoterol combined with three different steroids on ASM cell proliferation control.
Methods: Proliferation was determined by cell count 3 days after stimulation. Drugs were used at concentrations 1nM – 1microM. Protein expression (p21, p27) was determined by immuno-blot and -staining. Cyclic AMP was inhibited by DDA pre-incubation.
Results: Serum induced proliferation was reduced by steroids (dexamethasone, fluticasone, budesonide) in ASM cells of healthy controls but not of asthma patients. Formoterol dose dependently reduced cell proliferation by maximal 25% in both asthma and control ASM cells and this was paralleled by p27(Kip) activation in asthma ASM cells only. In combination the inhibitory effect of formoterol increased to 52% by dexamethasone, to 69% by budesonide and to 76% by fluticasone in healthy cells. In asthmatic ASM cells the combinded rug's effect was 47%, 52%, an 56% respectively. Inhibition of proliferation of asthmatic ASM cells by formoterol occurred through cAMP and p27(Kip), which were both increased by the addition of steroids. The anti-proliferative effect of the combined drugs in control ASM cells involves p21(Waf1) and in asthmatic cells by p27(Kip).
Conclusion: Formoterol activates a disease specific cAMP dependent anti-proliferative signalling in asthmatic ASM cells which is supported by the addition of steroids.

P4835

Synergistic effects between glycopyrronium bromide and indacaterol on a muscarinic agonist-induced contraction in airway smooth muscle

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Rationale: Bronchodilators play an important role in therapy for stable chronic obstructive pulmonary disease (COPD). Anticholinergics and $beta_2$-adrenoceotor agonists are widely used to improve lung function, symptoms, and QOL of patients with COPD. This study was designed to investigate whether combination of anticholinergics and $beta_2$-adrenoceptor agonists is beneficial. Combined effects on airway smooth muscle contraction were examined using glycopyrronium bromide (GB), a long-acting muscarinic antagonist (LAMA), and indacaterol, a long-acting $beta_2$-adrenoceptor agonist (LABA).
Methods: For record of isometric tension, the strips of tracheal smooth muscle of guinea pigs were placed in the organ bath and were perfused with the physiological solution at a constant flow rate of 2 ml/min.
Results: One nM indacaterol caused a modest (7.9%, n=18) inhibition of 1 μM methacholine (MCh)-induced contraction of tracheal smooth muscle. GB at 3, 10, and 30 nM caused a concentration-dependent inhibition of 1 μM MCh-induced contraction with values of percent inhibition of 11.1, 21.9, and 52.2%, respectively (each n=6). However, when equi-molars of GB were applied in the presence of 1 nM indacaterol, the inhibitory effects of GB (3, 10, and 30 nM)/indacaterol (1 nM) combination were markedly enhanced, with values of percent inhibition of 25.6 ($p<0.05$), 46.1 ($p<0.01$), and 91.2% ($p<0.01$), respectively (each n=6).
Conclusions: Indacaterol synergistically potentiated GB-induced relaxation against cholinergic stimulation in airway smooth muscle. These results may underlie the clinical benefit of combination therapy of LABA and LAMA for patients with COPD.

P4836

Cyclic AMP mediates the anti-asthma properties of the lidocaine analog JMF2-1

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Inhalation of JMF2-1, an analog of lidocaine with reduced anesthetic activity, prevents airway contraction and lung inflammation in experimental asthma models. We sought to test if JMF2-1 effects are a consequence of increased intracellular cAMP levels in asthma cell targets, such as smooth muscle cells and T cells. Apoptosis of T cells treated with JMF2-1 *in vitro* was assessed by flow cytometry. The spasmolytic effect of JMF2-1 was tested on isolated rat tracheal rings. Intracellular levels of cAMP from T cells and airway smooth muscle cells treated with JMF2-1 were detected by radioimmunoassay. We found that JMF2-1 inhibited tracheal ring contraction induced by carbachol. The antispasmodic effect remained unaltered following epithelium removal or pretreatment with L-NAME, but it was clearly sensitive to 9-(tetrahydro-2-furyl) adenine (SQ22,536), an adenylate cyclase inhibitor. JMF2-1 induced apoptosis of anti-CD3 activated T cells in a mechanism sensitive to zIETD, indicating that JMF2-1 mediates caspase-8-dependent apoptosis. JMF2-1 significantly increased cAMP intracellular levels ($P < 0.05$) of cultured airway smooth muscle cells (from 2.3±0.7 to 44.1±1.9) and T lymphocytes (from 36.3±5.9 to 208.3±25.8 pMol/mg of protein) (mean ± SEM, n=3). This effect was consistently abrogated by SQ22,536 and reproduced by forskolin in both systems. Our results suggest that JMF2-1 inhibits respiratory smooth muscle contraction as well as T cell survival through enhancement of intracellular cAMP levels. These findings may help to explain the anti-inflammatory and antispasmodic effects of JMF2-1 observed in previous studies.

P4837

Evidence for a non-β_2-adrenoceptor (β_2AR) binding site in human lung tissue for the long acting β_2-agonist (LABA) vilanterol

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Background: Vilanterol (VI) is a novel LABA with inherent 24 hour activity in development for inhaled once daily administration in combination with an inhaled corticosteroid for both COPD and asthma. We describe here an additional binding site in human lung tissue distinct from the orthosteric β_2AR binding site in an *in vitro* binding assay.
Methods: Radioligand saturation and competition binding experiments were performed by filtration with [^{3}H]VI or [^{3}H]propranolol and human lung parenchyma membranes at 37°C (+ 100μM Gpp(NH)p with NSB 10μM β_2AR antagonist ICI118551). Competition binding with a range of unlabelled β_2AR agonist/antagonists was determined (data shown mean ± SEM, n≥4). Statistical differences measured by ANOVA (Bonferroni post-test) with $P < 0.05$ deemed significant.
Results: Saturation binding data were best fitted to a one affinity site model with pK_D values 8.8±0.3 and 9.0±0.1 and B_{max} values 0.5±0.1 and 0.4±0.1 pmol/mg for [^{3}H]VI and [^{3}H]propranolol, respectively. ICI118551, propranolol, salbutamol, formoterol and carmoterol resulted in inhibition of [^{3}H]VI (~0.3nM) binding

to levels significantly different from VI. Indacaterol and salmeterol resulted in inhibition of [^{3}H]VI (~0.3nM) binding to levels not significantly different from VI. Indacaterol and VI resulted in inhibition of [^{3}H]propranolol (~1.7nM) binding to levels not significantly different from ICI118551.
Conclusions: VI exhibits a high affinity for the orthosteric β_2AR binding site in human lung parenchyma membranes. In addition, VI binds to an additional binding site, distinct from the functional orthosteric β_2AR binding site shared with salmeterol and indacaterol.

P4838
Small impact of mild and moderate renal impairment on the pharmacokinetics of inhaled NVA237
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Introduction: NVA237 (glycopyrronium bromide) is a long-acting muscarinic antagonist for the treatment of COPD. It is primarily eliminated by the kidneys. Since renal function decreases in elderly and the target population of NVA237 includes elderly patients it is important to investigate the effect of renal impairment (RI) on NVA237 pharmacokinetics.
Methods: Subjects: 8 with mild RI, 8 with moderate RI, 8 with severe RI, 6 with end-stage renal disease requiring dialysis (ESRD) and 18 demographically matching healthy volunteers (HV). Renal function was assessed by the estimated glomerular filtration rate (eGFR).
Doses: Single 100 μg dose of NVA237 delivered via the Breezhaler® device. ESRD subjects received a single dose on two occasions, between two dialysis sessions and at start of a 4-hr dialysis.
Results: A moderate increase in NVA237 total systemic exposure (AUClast) of up to 1.4-fold (on average) was seen in subjects with mild and moderate RI as compared to HVs. An increase of up to 2.2-fold was observed in subjects with severe RI and ESRD. Renal clearance (CLr) of NVA237 was strongly correlated with the degree of RI. In subjects with severe RI, CLr was reduced by about 80% compared with HVs. NVA237 was partially cleared during hemodialysis with an extraction ratio of 24.3%. NVA237 was well tolerated by HVs and RI subjects.
Conclusion: RI had an impact on NVA237 total systemic exposure which was moderate in subjects with mild and moderate RI (eGFR $\geq$ 30 mL/min/1.73m^2). The limited effect of severe RI on systemic exposure to NVA237 and the correlation analysis of total systemic clearance versus eGFR suggests that non-renal clearance mechanisms play a role in the elimination of NVA237.

P4839
Efficient deposition and sustained lung concentrations of NVA237 after inhalation via the Breezhaler® device in man
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Introduction: This study in healthy volunteers (HVs) was designed to investigate the bioavailability of inhaled NVA237 (glycopyrronium bromide-GP) delivered via the Breezhaler® device and to investigate the contributions of pulmonary and gastrointestinal (GI) absorption to systemic exposure. GI absorption was blocked with oral activated charcoal (AC) to study the pharmacokinetics (PK) of the NVA237 absorption via the lung.
Methods: In Part 1, NVA237 400 μg was administered orally to 10 HVs with and without concomitant administration of AC to demonstrate that GI absorption of NVA237 can be blocked. In Part 2 (n=20 HVs) the PK of 200 μg inhaled NVA237 with and without AC were compared to those of an i.v. infusion of 120 μg GP. Plasma PK data were analyzed by non-compartmental and compartmental methods.
Results: Result of Part 1 showed that oral AC was effective in blocking the oral absorption of NVA237. Absolute bioavailability of orally administered NVA237 (without AC) was estimated to be about 5%. In Part 2 the absolute bioavailability of inhaled NVA237 was about 40%. About 90% of systemic exposure was due to pulmonary absorption, 10% to GI absorption. About 36% of the inhaled dose was deposited and absorbed in the lungs. The mean terminal half-life of NVA237 was 52.5 h and 57.2 h in the inhalation treatments, 6.2 h after the i.v. dose and 2.8 h after the oral dose.
Conclusion: NVA237 inhaled via the Breezhaler® device is efficiently deposited and absorbed in the lungs. The terminal half life of NVA237 is much longer after inhalation than after i.v. or oral dosing which points to sustained lung concentrations of NVA237 following inhalation.

P4840
Guaifenesin suppresses MUC5AC content and secretion in human airway epithelial cell cultures: Comparison with N-acetylcysteine and ambroxol
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Background: Airway mucus hypersecretion is common in many respiratory diseases such as upper respiratory tract infections, asthma and COPD (chronic bronchitic form). Drugs to inhibit mucus production are needed. We showed that guaifenesin (glyceryl guaiacolate ether, GGE) inhibits unstimulated MUC5AC production in differentiated human airway epithelial cells *in vitro* (Seagrave *et al*. Exp Lung Res 2011;37:606–14).
Aims: We examined the effects of GGE on MUC5AC production and secretion in cells stimulated with IL-13 to produce additional mucus and compared the GGE effects with those of the mucolytic N-acetylcysteine (NAC) and the mucoactive drug ambroxol (Amb).
Hypothesis: GGE would inhibit IL-13-stimulated MUC5AC production.
Methods: Cultures were pre-treated with 1 ng/mL IL-13 for 3d, then treated with 10, 30, 100 or 300 μM GGE, NAC or Amb for 3, 8 or 24h in the presence of IL-13. The apical surfaces were washed and cells were lysed. MUC5AC content in the washes (secretion) and lysates (content) was assessed by ELISA.
Results: IL-13 increased MUC5AC secretion (2-fold) and content (1.5-fold) over baseline at 24h. GGE significantly inhibited MUC5AC secretion and content in a concentration-dependent manner, with IC_{50}s of ~110 and 150 μM and inhibition at 300 μM GGE of 80 and 90% respectively at 24h. Although NAC and Amb inhibited IL-13-induced MUC5AC production, inhibition was only 20–40% at 24hr and not concentration-dependent. Cell viability was not affected by any treatment.
Conclusions: GGE inhibits stimulated MUC5AC production (more effectively than NAC or AMB), suggesting that GGE may be useful in treating airway mucus hypersecretion.

P4841
Guaifenesin alters mucus rheology and improves mucociliary transport in human airway epithelial cell cultures: Comparison with N-acetylcysteine and ambroxol
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Background: Mucociliary transport (MCT) contributes to airway mucus and irritant clearance. Respiratory infections and inflammation (e.g. asthma & chronic bronchitis) can cause mucostasis-associated pathology. We showed that guaifenesin (glyceryl guaiacolate ether, GGE) decreases unstimulated mucus viscoelasticity and increases MCT in human differentiated human airway epithelial cells *in vitro* (Seagrave *et al*. Exp Lung Res 2011;37:606–14).
Aims: In cells treated with IL-13 to increase mucus, GGE was compared with N-acetylcysteine (NAC: mucolytic) and ambroxol (Amb: mucoactive).
Hypothesis: GGE reduces stimulated mucus viscoelasticity and increases MCT.
Methods: Cells were treated with 1 ng/mL IL-13 for 3d, then with 30 or 100 μM GGE, NAC, or Amb (+IL-13). At 3, 8, or 24h after treatment, MCT rates were analyzed from videos. Apical secretions from 8 and 24h were analyzed by micro parallel plate rheology.
Results: At 24h, IL-13 decreased MCT rates by >75% but did not affect G' (elastic) or G" (viscous) moduli. All drugs significantly increased MCT and decreased G' vs. IL-13 alone. GGE (30 μM) increased MCT >6-fold at 24hr and reduced G' to 10% of IL-13-only values. NAC reduced G' but increased MCT ~2-fold at 30 μM and decreased MCT to below baseline at 100 μM. Effects of Amb were smaller. The reduced MCT for NAC at 100 μM could be due to uncoupling of ciliary beat from mucus. Bonferroni analysis ($p<0.05$) showed significant advantages for GGE vs. NAC & Amb (MCT) and vs. Amb (rheology, G').
Conclusions: GGE increases MCT via decreased mucus viscoelasticity, which may be a useful property in treating certain respiratory diseases.

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The safety, tolerability and pharmacokinetics of AZD5069, a novel CXCR2 antagonist, in healthy Japanese volunteers
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Background: AZD5069 is a reversible antagonist at the human CXC chemokine receptor-2, with potential as an oral treatment of inflammatory diseases such as COPD. It has previously only been administered to Caucasian healthy volunteers and patients.
Methods: This was a Phase I, randomised, double-blind, placebo-controlled, single-centre, 6 cohort study in healthy Japanese males (n=63; 22–39 yrs). Subjects

received a single dose of AZD5069 (10–120 mg) on Day 1 (n=42), then twice-daily doses of AZD5069 (10–80 mg) for 7 days and a single dose on the last dosing day (n=36), or placebo (n=21). The safety and tolerability of AZD5069 was assessed primarily, with pharmacokinetics assessed by non-compartmental analysis.
Results: AZD5069 ≤80 mg twice-daily was well-tolerated with an acceptable safety profile. Expected clinically significant changes in laboratory safety parameters led to study withdrawals due to low blood neutrophil levels and elevated high sensitivity-C-reactive protein (hs-CRP) levels, meeting stopping criteria. Circulating neutrophil levels decreased with increasing plasma AZD5069 concentrations, but were recovering by 12 hrs post-dose and had returned to near normal at follow-up, 7–10 days post-last dose. AZD5069 was rapidly absorbed and AUC and C_{max} increased dose-proportionally with single and multiple doses. Steady-state was reached within 2–3 days following twice-daily dosing, with no, or minor, drug accumulation.
Conclusions: AZD5069 was well-tolerated. Decreases in neutrophil counts and increases in hs-CRP levels were observed as expected. No safety concerns were identified to preclude future evaluation. Systemic exposure to AZD5069 was dose proportional.

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Combining corticosteroids and NK1R antagonists: A new drugs combination to treat allergic diseases

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Introduction: Recently, using the rat mast cell/basophil cell line RBL-2H3, a major model system for the study of FcεRI intracellular signaling pathways, we found that optimal pharmacological blocking of autocrine activation of the neurokinin-1 receptor (NK1R) in response to FcεRI clustering suppresses antigen-induced 50% of maximal cell degranulation, and decreases by nearly 50% antigen-induced maximal cell activation.
Aim: To determine whether combining corticosteroids and NK1R antagonist may be a powerful therapeutic combination to control IgE-FcεRI complex responses in allergic diseases.
Methodology: IgE-sensitized RBL-2H3 cells were incubated with various concentrations of corticosteroids in combination or not with NK1R antagonist prior to FcεRI clustering. Cells degranulation and cysteinyl-leukotrienes (Cys-LTs) production were examined.
Results: Maximal concentrations of respective corticosteroids decreased by nearly 50% allergen-induced maximal degranulation and Cys-LTs production in basophils. Pharmacological blocking of NK1R alone has also produced similar inhibitory effects in basophils. Interestingly, the combination of corticosteroids and NK1R antagonist improved both time response and concentration efficacy of corticosteroids with nearly total inhibition of basophil allergic responses.
Conclusion: Combining corticosteroids and NK1R antagonist (patent WO2007/096782) is a promising therapeutic combination to increase corticosteroids efficacy while decreasing effective doses, and may give a second "breath" to corticosteroids patents that are no longer protected.
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Advantageous toxicity profile of an inhaled GATA-3-specific DNAzyme intended for anti-inflammatory treatment of Th2-driven asthma

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DNAzymes are single-stranded catalytic DNA molecules that specifically bind and cleave target mRNA sequences. Their potential as novel therapeutic agents has been demonstrated in a variety of disease models. However, no studies have yet addressed their toxicology and safety pharmacology profiles in detail. We have developed and characterized the human GATA-3-specific DNAyzme hgd40 intended for the treatment of Th2-driven asthma. Here we report results of a detailed toxicological analysis of inhaled hgd40. Subacute toxicity, immunotoxicity, and respiratory, cardiovascular, and CNS safety pharmacology were analyzed in rodents and non-rodents, and genotoxicity was assessed in human peripheral blood. Overall, hgd40 was very well tolerated when delivered by aerosol inhalation or slow intravenous infusion. Only marginal reversible histopathological changes were observed in the lungs of rats receiving the highest dose of inhaled hgd40. The changes consisted of slight mononuclear cell infiltration and alveolar histiocytosis, and moderate hyperplasia of bronchus-associated lymphoid tissue. No local or systemic adverse effects were observed in dogs. No compound-related respiratory, cardiovascular, or CNS adverse events were observed. The only relevant immunological findings were very slight dose-dependent changes in interleukin-10 and interferon-γ levels in bronchoalveolar lavage fluid that may represent pharmacological activity of hgd40. Taken together, these results support the direct delivery of the GATA-3-specific DNAzyme hgd40 via inhalation for the treatment of asthma in subsequent clinical studies.