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, PEF 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 stimuli. Steroids and β2 agonists decrease airway contractility and inhaled budesonide reverses 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. To assess the effect of steroids, slices were preincubated with budesonide (Bud) for 1 h prior to IL-13. Data shown are mean ± S.E.M. of baseline luminal area ± SEM. IL-13 significantly increased bronchoconstriction to a maximal effective concentration (100 μM) of Cch (Control: C: -80 ± 13, IL-13: -89 ± 20, p=0.02) and decreased the effect of β2 agonists (Form: 2.5 ± 1.2 vs. 0.7 ± 0.2, p=0.01). Bud significantly decreased the AHR to Cch following IL-13 (IL-13: -93 ± 8, IL-13+Bud: -80 ± 13, p=0.01), but had little effect on IL-13-induced impairment of the Form response (IL-13: -20 ± 13, IL-13+Bud: 28 ± 17, p=0.3). In contrast, bronchodilation to 100 μM Fak was rescued by Bud (IL-13: 42 ± 8, IL-13+Bud: 82 ± 8, p<0.01). Cch: 76 ± 5, IL-13/Bud: 82 ± 8, p<0.01).

These data suggest that pretreatment with budesonide completely prevents the effect 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 Tumor 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 liquid clearance and lung function in a porcine model of ALI.

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” of the two IgE receptors on IgE-induced airway remodelling, and c) the potential of the two IgE receptors on IgE-induced airway remodelling.

Methods: 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 male subjects 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 has been demonstrated in various studies in rodents. AP301 has also 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. Pre-clinical safety assessment, drug, 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 ± 3.8 pA to 22 ± 5.3 pA, IL-13: 20 ± 8, p<0.05. (C: 76 ± 5, IL-13/Bud: 82 ± 8, p<0.01).

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.
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 1, II 1, 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, \( p < 0.05 \)). 4h post-BD drop in hyperinflation at FRC was positively associated with the degree of hyperinflation (r=0.4, \( p < 0.05 \)). A drop in pre-bronchodilation hyperinflation was observed 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 asthmatic 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 1 nM – 1 micromolar. Protein expression (p27, p21) was determined by immuno-blots 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 asthmatic 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 asthmatic 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 combined 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 glycopyronium bromide and indacaterol on a mucociliary 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-adrenoceptor 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 glycopyronium bromide (GB), a long-acting muscarinic agonist (LAMA), and indacaterol, a long-acting beta-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 \( \mu \text{m} \) methacholine (MCh) induced contraction of tracheal smooth muscle. GB at 3, 10, and 30 nM caused a concentration-dependent inhibition of 1 \( \mu \text{M} \) MCh-induced contraction with values of percent inhibition of 11, 1, 21, 9, and 52%, 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. JMF2-1 reduced airway smooth muscle 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 antisuppressive effect remained unaltered following epithelium removal or pretreatment with L-NAMe, but it was clearly sensitive to (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 ZEtD, indicating that JMF2-1 mediates caspase-8 dependent apoptotic pathways. JMF2-1 significantly increased cAMP intracellular levels in asthma cell targets, such as smooth muscle cells and T cells. Activation of ASM cells and T cells 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 asthmatic 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 asthmatic 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 combined 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.

P4837
Evidence for a non-\( \beta_2 \)-adrenoceptor (\( \beta_2 \AR \)) binding site in human lung tissue for the long acting \( \beta_2 \)-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 \( \beta_2 \AR \) antagonist. Methods: Radioligand saturation and competition binding experiments were performed with [\( ^3 \text{H} \)]VI or [\( ^3 \text{H} \)]propranolol and human lung parenchyma membranes at 37°C (+ 100 μM GppNHz with NSB 10 μM \( \beta_2 \AR \) antago-

nist ICI18551). Competition binding with a range of unlabelled \( \beta_2 \AR \) ago-
nist/agonists 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 \( K_D \) 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 [\( ^3 \text{H} \)]VI and [\( ^3 \text{H} \)]propranolol, respectively. ICI18551, propranolol, salbutamol, formoterol and carmoterol resulted in inhibition of [\( ^3 \text{H} \)]VI (~~0.3nM) binding.
to levels significantly different from VI. Indacaterol and salmeterol resulted in inhibition of [3H]VI (1.0 nM) binding to levels not significantly different from VI. Indacaterol and VI resulted in inhibition of [3H]Propranolol (1.0 nM) binding to levels not significantly different from IC50. 

Conclusions: VI exhibits a high affinity for the orthosteric β2AR binding site in human lung parenchyma membranes. In addition, VI binds to an additional binding site, distinct from the functional orthosteric β2AR binding site shared with salmeterol and indacaterol.

P4838 Small impact of mild and moderate renal impairment on the pharmacokinetics of inhaled NV A237

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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 NV A237 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 NV A237 total systemic exposure (AUClast) of ≥30 mL/min/1.73 m2. The differences in AUClast were smaller. The reduced MCT for NAC at 100 μM GEE compared with IL-13-only values. NAC reduced G' but increased MCT and GEE compared with IL-13.

Conclusions: We showed that guaifenesin reduces stimulated mucus viscoelasticity and increases MCT in human differentiated human airway epithelial cells in vitro. Further studies are required to evaluate the in vivo effects and clinical relevance of these findings.

P4840 Guaifenesin suppresses MUC5AC content and secretion in human airway epithelial cell cultures: Comparison with N-acetylcysteine and ambroxol

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Hypothesis: GEE would inhibit IL-13-stimulated MUC5AC production.

Methods: Cells were treated with 1 ng/mL IL-13 for 3d, then with 30 or 100 μM GEE or 100 μM NAC and 30 or 100 μM Amb for 24h after IL-13.

Results: GEE significantly inhibited MUC5AC production compared with IL-13 only. NAC reduced GEE to below baseline at 24h. Amb reduced MUC5AC production. MCT for NAC and Amb was similar.

Conclusions: GEE inhibits stimulated MUC5AC production (more effectively than NAC or Amb), suggesting that GEE may be useful in treating airway mucus hypersecretion.
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 potential 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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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 DNAzyme 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.