95. Translational models of disease

P840

Transient receptor potential ankyrin 1 (TRPA1)-mediated cough

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TRPA1 detects many endogenous inflammatory and environmental pollutant molecules. Previous studies implicated TRPA1 as a drug target for antitussive therapy. We evaluated relative efficacy of the TRPA1 activation in inducing cough in guinea pigs. Inhalation of the TRPA1 agonist allyl-isothiocyanate (AITC) evoked cough with a maximally effective concentration of 10mM (2.8±0.8 coughs/5min above vehicle, n=12) that was abolished by the TRPA1 selective antagonist AP-18 (1mM, n=8). AITC was ~3-times less effective in inducing cough than the TRPV1 agonist capsaicin in the sub-maximally effective selective concentration (50μM) (8.2±2.1 coughs/5min above vehicle, n=9, $p<0.05$). Ex vivo single fiber recordings showed that AITC was ~3-times less effective than capsaicin in evoking sustained activation of the cough-triggering tracheal jugular C-fibers (65±18 [n=7] vs. 210±57 [n=7] maximum action potentials/30s bin, $p<0.05$). AITC failed to activate the capsaicin-insensitive tracheal nodose A-fibers (n=6). Another TRPA1 agonist cinnamaldehyde (10mM) was surprisingly ~2-fold more effective than AITC in inducing cough (6.0±0.9 coughs/5min above vehicle, n=12, $p<0.01$). The cinnamaldehyde (10mM)-induced cough was only partially (by~60%, n=8) inhibited by the TRPA1 antagonists AP-18 (1mM), but abolished by combination of AP-18 and the TRPV1 antagonist I-RTX (30μM, n=6). We conclude that in guinea pigs TRPA1 initiates cough that is relatively modest compared to cough initiated by TRPV1. This is likely due to lower efficacy of TRPA1 in inducing sustained activation of the cough-triggering C-fibers. Our data indicate that TRPV1 in addition to TRPA1 contributes to cough evoked by cinnamaldehyde in this species.
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P841

Toxicity and safety of dextromethorphan inhalation in a mouse model prior its use in humans

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Background: Dextromethorphan (DM) is a non narcotic codeine analogue, widely used as antitusive agent. DM is active mostly centrally, but also in lung regions. DM was shown to be effective when given by inhalation in guinea pigs. Oral DM prior bronchoscopy is effective in reducing cough and dyspnea. The use of inhalation of DM could lead to faster beneficial effect of the drug, and to avoidance of oral preparation prior bronchoscopy.
Objectives: To define the toxicity of inhaled DM in a mouse model, using different doses and comparison with sham inhalation.
Methods: Female BALB/c mice, were divided to 4 groups exposed to increasing concentrations of DM solution: normal saline, 60, 90 and 360 mg/kg. Inhalation studies lasted ~ 20 min/day, for 3 weeks. Blood analysis and complete histological evaluation were performed.
Results: There was no evidence of toxic effect in blood biochemical parameters, complete blood counts and gross pathology. Histological evaluation of the heart, kidneys, spleen, liver and pancreas was normal in all groups. However, abnormalities were found on lung pathology. In the high concentration group diffuse alveolar damage, alveolar hemorrhage, pulmonary congestion and severe bolus emphysema were shown, whereas in the low dose group only very mild emphysema was found. It is noteworthy that no signs of morbidity or behavioral changes were observed in all groups.
Conclusions: Very high dose Dextromethorphan inhalations have no biochemical or hemathological toxic effects, but may be toxic to the lungs. However, inhalation dose of 60 mg/kg seems to be safe. These data suggest that treatment with inhaled DM in lower doses may be safe and can be used in humans.

P842

Non-genomic effect of glucocorticoids on inhibition of expression of CD63 on peripheral blood basophils

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Background: Glucocorticoids (GC) could inhibit histamine release from rat peritoneal mast cells within 10 minutes, which classical genomic mechanism could not explain. The clinical efficacy of inhaled corticosteroids combined with long-acting beta 2-agonists has been widely demonstrated in asthma.
Objective: In order to validate the benefit of the combined these agents in vitro, we studied a rapid effect of fluticasone propionate (FP) and formoterol (FORM) alone and in-combination on inhibition of expression of CD63, which is a maker of degranulation of basophils, in house dust mite (HDM)-sensitive patients with asthma.
Methods: Whole peripheral bloods from asthmatic patients were incubated with HDM for 20 min in the pretreatment of FP and/or FORM for 1 hour. We assessed the expression of CD63 on basophils by quantifying the mean fluorescence of CD63 in IgE-positive cells by FACS analysis.
Results: A high concentration of FP (10^{-8}M) alone, but not a low concentration of FP (10^{-12}M) alone, inhibits CD63 expression-induced with HDM. The combination of FP (10^{-8}M) and FORM (10^{-7}M) more inhibits CD 63 expression on basophils.
Conclusion: The study provided evidence that non-genomic mechanism might be involved in rapid effect of glucocorticoids on basophils in asthma.

P843

Expression and functionality of P-glycoprotein in human bronchial epithelial cells *in vitro*

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P-glycoprotein (P-gp) is expressed in normal tissues with barrier functions where it participates in cell defence mechanisms (Huls, M. *et al.* J Pharm Exp Ther 2009; 328:3-9). Its presence in the bronchial epithelium and role in lung protection against inhaled toxicants has yet to be elucidated.
The human bronchial epithelial cell line Calu-3 and normal human bronchial epithelial (NHBE) cells were cultured at an air-liquid interface on Transwell® inserts for 21 days. P-gp expression was evaluated by quantitative polymerase chain reaction and its functionality was assessed by permeability measurements using the established substrate ^{3}H-digoxin either alone or in the presence of chemical or biomolecular inhibitors.
P-gp was absent in NHBE cells and moderately expressed in Calu-3 cells. Net secretory transport of ^{3}H-digoxin was observed in both models. This was reduced at 4°C and in the presence of the selective but non specific P-gp inhibitor PSC833 and the multidrug resistance protein (MRP) inhibitor MK571. The P-gp specific antibody inhibitor UIC2 and the metabolic inhibitors sodium azide and sodium dichloroacetate had no effect on ^{3}H-digoxin transport in Calu-3 cells.
The presence of active transport mechanisms in cultures of human bronchial epithelial cells was demonstrated, although they differed between the models tested. P-gp was not detected in NHBE cells, in line with observed low gene expression in human lung tissue (Bleasby, K. *et al.* Xenobiotica 2006; 36:963-988). The involvement of P-gp could not be confirmed and the transporter(s) responsible for ^{3}H-digoxin asymmetric broncho-epithelial permeability remain(s) to be identified.

P844

Fluticasone furoate, a novel corticosteroid, maintains glucocorticoid receptor nuclear localisation for 24 hours after washout in monocytes

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Background: Fluticasone furoate (FF) is a novel corticosteroid (CS) under development for inhaled once daily administration for chronic obstructive pulmonary disease and asthma. CS act via binding to the glucocorticoid receptor (GR). Upon activation, GR translocates into the nucleus, an essential prerequisite for CS function. In dose-ranging studies in asthmatics, FF had 24 hour (h) duration of efficacy. We hypothesized, therefore, that the sustained activity of FF is due to prolonged GR nuclear translocation.
Method: The effects of FF on GR nuclear translocation over a 24h time-course was examined in U937 monocytes. In addition, we compared the effect of a 20h washout on GR nuclear localisation following treatment with FF for 4h. Statistical analysis was performed using Kruskal-Wallis analysis and results represented as mean±SEM.
Results: FF significantly induced GR nuclear translocation in a time- (2-24hr) and concentration- (10^{-11}–10^{-7}M) dependent manner ($p<0.05$ for each time and concentration measured compared to unstimulated controls). FF (10^{-7}M) significantly increased nuclear GR levels at 4h (5.4±0.57 fold increase, $p<0.05$) which was maintained at 16 and 24h. There was a corresponding decrease in cytosolic GR over this time scale. Importantly, in the washout experiments, there was a similar level of GR nuclear translocation at 24h after 4h FF (10^{-7}M) treatment as seen with continual FF exposure (5.7±0.85 versus 6.6±0.92 fold increase, p=ns).
Conclusions: FF induced GR nuclear translocation in a time and concentration dependent manner. Exposure of cells to FF for 4h was as effective as continued exposure for 24h.
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P845

Roflumilast N-oxide, a selective PDE4 inhibitor, curbs platelet-leukocyte interactions

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Objective: COPD is associated with cardiovascular comorbidities. Platelet (Plt)–leukocyte interactions play a role in vascular disease. We tested the effect of the PDE4 inhibitor roflumilast N-oxide (RNO), the active metabolite of roflumilast approved for severe COPD in EU, on Plt-mediated neutrophil (Neu) recruitment and tissue factor (TF) expression in monocytes (Mn).
Methods: Neu adhesion to spread Plt or endothelium (EC) and Plt-induced TF in Mn were analysed with RNO with or without formoterol (F) (100 nM).*$p<0.05$ vs control (C).
Results: In a flow adhesion assay RNO, alone or with F, concentration-dependently reduced the number of Neu firmly adhered on spread Plt at 10 dynes/cm^2 shear stress (C 106±18 and RNO [100nM] 71±7*; F 110±11 and RNO&F 58±7* Neu per field). RNO also inhibited P-selectin (C 59±11, RNO 35±7*, F 65±13, RNO&F 16±4* Neu per field) and Plt-induced (C 49±2, RNO 12±2*, F 50±11, RNO&F 2±0* Neu per field) Neu recruitment on EC. Src kinases and downstream effectors Pyk2 and PI(3)K mediate P-selectin-triggered PSGL-1-Mac-1 cross-talk. RNO curbed Src-mediated Pyk2 phosphorylation and PI3K-mediated Akt phosphorylation, while inducing phosphorylation of Csk, the major negative regulator of Src, by protein kinase A. In Mn exposed to activated Plt (24h), RNO dose-dependently reduced TF activity (C 67±7, F 56±6, RNO [100nM] 9.7±3.9*, RNO&F 2±0.8*, arbitrary units). In the presence of F, RNO at 1nM reduced TF activity to 24.5±7.8*. RNO (100nM) also reduced TF mRNA (C 72±10, RNO 9±3 fold increase).
Conclusion: The PDE4 inhibitor RNO curbs Plt–leukocyte interactions.

P846

A-kinase anchoring proteins as novel regulators of airway smooth muscle function

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Chronic obstructive pulmonary disease (COPD) is a chronic inflammatory disease mainly caused by cigarette smoking (CS). Compartmentalization of cAMP signaling regulates cellular responses to (local) changes in cAMP levels. In this respect, A-kinase anchoring proteins (AKAPs) compartmentalize cAMP signaling by differentially docking proteins involved in cAMP signaling, including β2-adrenoceptors and the cAMP effectors PKA and Epac. Interestingly, AKAP79 (aka AKAP5) is involved in β2-adrenoceptor desensitization, which is opposed by AKAP250 (aka AKAP12). Here we studied the expression pattern and functions of AKAPs in regulating inflammatory cytokine release and the responsiveness to β2-agonists.
AKAP79, AKAP250 and AKAP450 (aka AKAP9) are expressed in human airway smooth muscle (hASM). Treatment with CS downregulated AKAP250 and (to a lesser extent) AKAP79, but not AKAP450. In lung tissue of COPD patients all these AKAPs were downregulated. In hASM cells, CS-induced IL-8 release was dose-dependently decreased by the β2-agonist fenoterol as well as by direct activation of PKA and (only marginally) of Epac. CS-induced IL-8 release was augmented by the PKA-binding blocking peptide st-Ht31, a generic AKAP inhibitor. Importantly, in the presence of st-Ht31 fenoterol was unable to reduce CS-induced IL-8, whereas the PKA activator was still fully effective.
In conclusion, AKAPs are expressed in the airways and coordinate the communication between β2-adrenoceptors and cAMP effectors in order to reduce CS-induced inflammation. Since AKAP expression is altered in COPD, AKAPs could contribute to the pathophysiology of this disease. Supported by Stichting Astma Bestrijding and a Rosalind Franklin Fellowship.

P847

Bosentan is superior to ambrisentan in reducing the expression of asthma- and COPD-related cytokines

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TNFa substantially contributes to the establishment of chronic airway inflammation. TNFa-induced expression of inflammatory genes in human airway smooth muscle cells (HASMCs) might depend on endothelin-1 (ET-1) signaling.
We investigated inflammatory gene expression in TNFa-exposed HASMCs. We compared the anti-inflammatory effects of the non-selective endothelin receptor A (ETAR)/ETBR antagonist Bosentan with those of the ETAR-selective antagonist Ambrisentan.
The overall response of cultivated HASMCs of n=8 current smokers to TNFa in the absence or presence of Bosentan or Ambrisentan was investigated by gene expression analysis with the Agilent Whole Genome Oligo Microarray technique (40,000 genes). Results of Asthma- and/or COPD-related cytokines/chemokines were verified by quantitative RT-PCR and ELISA.
The expression of 4,948±316 genes was induced twofold or more by TNFα. Among them were GM-CSF, G-CSF, 8 CC and 8 CXC family members and 5 interleukins. Bosentan and Ambrisentan reduced the expression of 310±51 or 396±63 genes, respectively, in TNFα-exposed HASMCs. Among them were CCL2/5/7/8/19/20, CXCL6/10, CX3CL1, IL-6/7/23 and GM-CSF (all $p<0.05$). The release of CCL2, CCL7, CX3CL1 and GM-CSF was more efficiently reduced by Bosentan compared with Ambrisentan ($p<0.05$). With the exception of GM-CSF, the effects of ET receptor antagonists on these factors were due to inhibition of gene transcription.
HASMCs contribute to the establishment of chronic airway inflammation in asthma and COPD. Particularly none-selective ET receptor antagonists might have therapeutic utility in early stages of chronic airway diseases by counteracting the establishment of inflammatory processes.

P848

Simvastatin selectively inhibits TSLP-production in primary bronchial epithelial cells from COPD donors

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Background: Possibly reflecting anti-inflammatory properties, statin treatment may ameliorate COPD exacerbations. Viral infections apparently cause TH2 type COPD exacerbations. We have shown previously that thymic stromal lymphopoietin (TSLP), a cytokine linking innate and adaptive immunity and switching on TH2 type inflammation, is overproduced in viral stimulated epithelial cells from GOLD stage IV COPD donors.
Objective: Explore effects of simvastatin on viral-induced TSLP in epithelial cells from patients with GOLD stage II COPD.
Methods: Primary bronchial epithelial cells, obtained by fibre optic bronchoscopy from COPD (n=4) and healthy individual (n=3) donors, were grown in 12-well plates and stimulated with a synthetic viral surrogate, double-stranded RNA (dsRNA, 10μg/ml) to induce cytokine expression (3h, RT-qPCR) and production (24 h, ELISA). Simvastatin (0,2-5μg) with or without mevalonate (13μg/ml) was added 18 h prior to dsRNA. Alternatively, dexamethasone (1μM) was added 1 h prior to dsRNA.
Results: dsRNA induced TSLP, TNF-alpha and IL-8 mRNA and protein expression ($p<0.05$-$p<0.001$). Simvastatin dose-dependently, but not dexamethasone, inhibited dsRNA-induced TSLP mRNA expression and protein release ($p<0.05$-$p<0.01$) in COPD cells. Simvastatin acted independent of mevalonate. dsRNA-induced TNF-alpha and IL-8 were not inhibited ($p>0.05$).
Conclusion: Independent of the mevalonic pathway, simvastatin selectively inhibited dsRNA-induced TSLP-production in COPD cells. These data support exploration of statin treatment in viral-induced COPD exacerbations. The pharma-

cology of simvastatin may unravel paths of selective inhibition of TSLP-production in COPD epithelium.

P849

Effects of corticosteroid or montelukast associated to iNOS inhibitior on inflammation and remodelling of distal lung in animals with chronic inflammation

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Aims: Distal lung alterations have been recently addressed on asthma pathophysiology. We evaluated oxidative stress, actin content, IL5 and MMP9 positive cells in distal lung parenchyma after montelukast or dexamethasone treatments associated or not to an iNOS inhibitor (1400W) in guinea pigs (GP) with chronic inflammation.
Methods: GP were inhaled with ovalbumin (OVA group-2x/week/4weeks). After 4th inhalation, GP were treated with montelukast (M group-10mg/kg/PO/day) or dexamethasone (D group-5mg/kg/IP/day). 1400W (W group-1mg/kg/day) was given daily in the last 4 days (W, DW and MW groups). After 72 hrs of 7th inhalation, GP were anesthetised, lung strips were submitted to histopathological evaluation.
Results: Isoprostane was reduced in M (9.6±0.4%), D (7.4±0.2%), MW (6.1±0.1%), DW (5.5±0.2%) and W (6.9±0.4%) compared to OVA (14.5±0.2%, $p<0.05$). Actin content was attenuated in M (7.6±0.5%), D (6.3±0.2%), MW (6.2±0.4%), DW (5.4±0.2%) and W (5.7±0.2%) compared to OVA (9.6±0.5%, $p<0.05$). There was a decrease of IL5+ cells in W ($4.9\pm0.5/10^4$ μm^2), M ($6.2\pm0.3/10^4$ μm^2), D ($4.8\pm0.3/10^4$ μm^2), DW ($2.8\pm0.2/10^4$ μm^2), and MW ($5.0\pm0.3/10^4 \mu m^2$) compared to OVA ($8.2\pm0.5/10^4$ μm^2, $p<0.05$). There was also a reduction of MMP9+ cells in M ($6.4\pm0.4/10^4$ μm^2)), D ($5.4\pm0.3/10^4$ μm^2), MW ($5.8\pm0.4/10^4$ μm^2), DW ($6.2\pm0.5/10^4$ μm^2) and W ($4.8\pm0.4/10^4$ μm^2) compared to OVA ($9.6\pm0.5/10^4$ μm^2, $p<0.05$).
Conclusions: In this animal model, corticosteroid or montelukast associated to iNOS inhibitior contributes to the reduction of the oxidative stress, actin content, MMP9 expression and was efficient to attenuate Th2 cytokine expression in distal lung.
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P850

The effect of the different methanolic extracts of *nigella sativa* on tracheal responsiveness of guinea-pigs

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In previous studies, the relaxant, anticholinergic (functional antagonism) and antihistaminic effects of *Nigella sativa* have been demonstrated on guinea pig tracheal chains. Therefore in the present study, the relaxant effects of 5 different methanolic extracts (20%, 40%, 60%, 80% and 100% methanolic fractions) of *Nigella sativa* on tracheal chains of guinea pigs were examined. The relaxant effects of four cumulative concentrations of each fraction (0.8, 1.2, 1.6 and 2.0 g%) in comparison with saline as negative control and four cumulative concentrations of theophylline (0.2, 0.4, 0.6 and 0.8 mM) were examined by their relaxant effects on precontracted tracheal chains of guinea pig by 60 mM KCl (group 1) and 10 μM methacholine (group 2, n = 7 for each group). In group 1, all concentrations of 100% methanolic extract showed significant contractile effect on guinea pig tracheal chains ($p<0.05$ to $p<0.01$). In group 2, all concentrations of theophylline and methanolic extracts showed significant relaxant effects compared to that of saline ($p<0.05$ to $p<0.001$). The relaxant effect of different concentrations of al fractions were significantly greater in group 2 compared to group 1 experiments ($p<0.001$). There were significant positive correlations between the relaxant effects and concentrations for theophylline and all fractions in group2 ($p<0.05$ to $p<0.001$). These results showed a potent relaxant effect for 20% methanolic extract and weaker relaxant effect for other fractions from *Nigella sativa* on tracheal chains of guinea pigs.

P851

Effects of aclidinium on cigarette smoke-induced fibroblast activation *in vitro*

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Introduction: Lung fibroblast activation in chronic obstructive pulmonary disease (COPD) results in expression of α-smooth muscle actin (αSMA) and collagen type I. Cigarette smoke (CS) promotes lung fibroblast proliferation via cholinergic system activation.
Aims: To assess the effects of aclidinium bromide, a novel, long-acting muscarinic antagonist, on human lung fibroblast activation after CS exposure *in vitro*.
Methods: Lung fibroblasts were incubated with aclidinium (10^{-9}M-10^{-7}M), the ERK 1/2 inhibitor PD98059 (10μM), the cAMP analogue dbcAMP (1mM) or the antioxidants N-acetylcisteine (NAC; 1mM) and apocynin (100μM) for 30 min and then exposed to CS extract (CSE) for 48 h. Collagen type I, αSMA expression and ERK 1/2 phosphorylation were measured by RT-PCR and/or Western blot (WB). Intracellular reactive oxygen species (ROS) were measured by DCFDA fluorescence dye. Protein expression by the NADPH complex gp67phox and choline acetyltransferase (ChAT) were measured by WB.
Results: Aclidinium, PD98059, dbcAMP, NAC and apocynin attenuated the CSE-induced increase in αSMA and collagen type I expression. Aclidinium also attenuated the CSE-dependent increase in Phospho-ERK and the CSE-induced increase in ROS to 50% of control. NAC and apocynin fully suppressed the increase in ROS, and PD98059 and dbcAMP reduced it to 20% of control. Aclidinium 10^{-7}M completely suppressed the CSE-induced increase in gp67phox expression. A CSE-induced ChAT upregulation suggested an autocrine acetylcholine regulation in response to CSE.
Conclusions: Aclidinium reduces human lung fibroblast activation following CSE exposure *in vitro*. Aclidinium may reduce lung fibroblast activation in patients with COPD after CS exposure.

P852

Effects of aclidinium on airway remodelling in guinea pigs chronically exposed to cigarette smoke

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Introduction: Exposure to cigarette smoke (CS) causes airway remodelling and airflow obstruction; it is a main risk factor in chronic obstructive pulmonary disease.
Aims: To investigate whether aclidinium bromide, a novel muscarinic antagonist, reduces airway remodelling in guinea pigs chronically exposed to CS.
Methods: Male guinea pigs (n=46) were divided into 2 groups: control (n=22) and exposed to CS (n=24; 6 cigarettes/day, 5 days/week for 24 weeks). Animals received nebulised vehicle, aclidinium 10μg/mL or aclidinium 30μg/mL 60 min before CS exposure. The thickness of the adventitia, muscularis and mucosal layers of the airway wall was measured by planimetry in immunostained sections. Emphysema and goblet cell metaplasia were evaluated using sections stained with haematoxylin-eosin and alcian blue, respectively. The internal luminal perimeter of each airway served as a reference to normalise and stratify the assessments.
Results: Aclidinium prevented thickening of the small airway muscularis layer in animals exposed to CS. Thickness after CS exposure was: aclidinium 10μg/mL 18μm and aclidinium 30μg/mL 21μm vs vehicle 32μm ($p<0.05$ both doses). Aclidinium (both doses) reduced the amount of smooth muscle content (α-actin) in CS-exposed animals. Thickening of the adventitia and mucosal layers, goblet cell metaplasia and emphysema were not significantly reduced with aclidinium treatment.
Conclusions: Aclidinium 10μg/mL and 30μg/mL reduce airway remodelling in guinea pigs by reducing the muscularisation of small airways seen after chronic CS exposure.
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Effects of aclidinium on respiratory function in guinea pigs chronically exposed to cigarette smoke

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Introduction: Chronic obstructive pulmonary disease is characterised functionally by decreased respiratory function due to airflow obstruction.
Aims: To evaluate the effects of aclidinium bromide, a novel muscarinic antagonist, on respiratory function and signs of bronchial irritation in guinea pigs chronically exposed to cigarette smoke (CS).
Methods: Male guinea pigs (n=46) were divided into 2 groups: control (n=22) and CS-exposed (n=24, 6 cigarettes/day, 5 days/week for 24 weeks). Animals received vehicle, aclidinium 10μg/mL or aclidinium 30μg/mL for 6 months. Respiratory function was evaluated twice-weekly using the enhanced pause (Penh) parameter, before CS exposure (24 h after last exposure; baseline) and 10 min after CS exposure. Changes in Penh were assessed as the area under the curve. The number of cough episodes in the first minute post CS exposure and the number of respiratory crises were monitored throughout the study period.
Results: Exposure to CS increased Penh at baseline and 10 min after CS expo-

sure. Compared with CS-exposed non-treated animals, treatment with aclidinium 30μg/mL resulted in a lower increase in Penh after CS exposure (vehicle vs aclidinium 30μg/mL: 110.6±26.7 vs 71.4±32.4; p=0.016). CS exposure resulted in cough episodes and respiratory crises which were attenuated by aclidinium 30μg/mL.
Conclusions: In guinea pigs chronically exposed to CS, aclidinium 30μg/mL improved respiratory function by significantly reducing the increase in Penh after CS exposure and reducing the development of cough and respiratory crises.
This study was supported by Almirall S.A., Barcelona, Spain, and Consorcios Estratégicos Nacionales en Investigación Técnica (CENIT).

P854
Protective effect of lecithinized superoxide dismutase (PC-SOD) against elastase-induced pulmonary emphysema in mice
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No medication exists that clearly improves mortality of chronic obstructive pulmonary disease (COPD). Oxidative molecules, in particular superoxide anions would play important roles in the abnormal inflammatory response and in pulmonary emphysema, which arises due to an imbalance in proteases and antiproteases and increased levels of apoptosis. Superoxide dismutase (SOD) catalyses the dismutation of a superoxide anion to hydrogen peroxide. Lecithinized SOD (PC-SOD) has overcome a number of the clinical limitations of SOD, including low tissue affinity and low stability in plasma. In this study, we examine the effect of PC-SOD on elastase-induced pulmonary emphysema.
The severity of the pulmonary inflammatory response and emphysema in mice was assessed by various criteria, such as enlargement of airspace.
Inhalation of PC-SOD suppressed elastase-induced pulmonary inflammation, emphysema and dysfunction. Inhalation of PC-SOD also suppressed the elastase-induced increase in the pulmonary level of superoxide anions, cell death, activation of proteases and expression of pro-inflammatory cytokines and chemokines. We also found that inhalation of PC-SOD suppressed cigarette smoke-induced pulmonary inflammation.
The results suggest that PC-SOD protects against pulmonary emphysema by inhibition of inflammation and cell death and amelioration of the protease/antiprotease imbalance. We propose that inhalation of PC-SOD would be therapeutically beneficial for COPD.

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Prevention of adverse pulmonary consequences of myocardial ischaemia in rats
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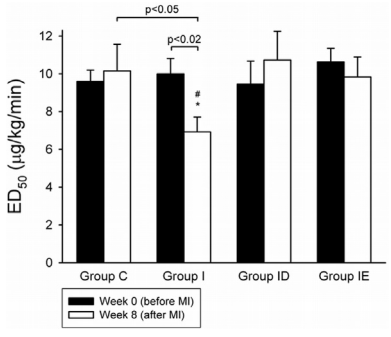
The efficiency of treatment strategies against airway hyperresponsiveness (AH) were compared following chronic postcapillary pulmonary hypertension induced by myocardial ischaemia (MI). Airway resistance (Raw) was measured in four groups of rats under baseline conditions, and following iv infusions of 2-18 μg/kg/min methacholine (MCh). Sham surgery was then performed in Group C, while the left interventricular coronary artery was ligated in the other groups without treatment (Group I), or daily treatments with combined angiotensin enzyme converter (ACE) inhibitor and diuretics (enalapril, lasix, Group IE), or a calcium channel blocker (diltiazem, Group ID). Eight weeks later, MCh provocations were repeated. Equivalent dose of MCh causing 50% increase in Raw (ED_{50}) was determined. Left atrial pressure (Pla) was estimated from the end-diastolic left ventricular pressure. Elevations in Pla to MI (6.8±1.1 [SD] vs. 15.2±1.3 mmHg in Groups C and I, respectively) were not affected by the treatment in Group ID (13.7±3.1 mmHg), whereas they were inhibited in Group IE (10.9±3.2 mmHg, p=0.005). The development of AH following MI was completely abolished in both Groups ID and IE.



These findings suggest the efficiency of combined ACE inhibitor and diuretics to protect the adverse pulmonary haemodynamical consequences of MI, while both treatment strategies have similar affinities to prevent AH.
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P856
Can streptomycin prevent the selection of isoniazid-resistant mutants in nude mice infected with *M. tuberculosis*?
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Rationale: In a previous study, the treatment of *M.tuberculosis* infected athymic nu/nu (nude) mice with the bactericidal drug isoniazid (H) combined with the two sterilizing drugs, rifampicin (R) and pyrazinamide (Z) failed and selected H-resistant mutants. We investigated whether the combination of H with the bactericidal drug, streptomycin (S) would be able to prevent that selection.
Methods: A total of 80 nude mice were aerosol infected with 3.67±0.13 log10 of *M. tuberculosis* H37Rv. Two weeks later, at treatment initiation, mice were randomized in the following subgroups: untreated and treated with RZH + ethambutol (E) as controls; treated with SH or RH, as tests, Treatment was given five days/week for 12 weeks. Drug doses (mg/kg) were 10 for R and H, 150 for Z and 100 for S and E. Lung CFU counts were done the day after infection, on treatment initiation, and after 4, 8 and 12 weeks of treatment on plain and H 0.2 μg/ml-containing 7H11 selective plates.
Results: Lung CFU counts were 7.40±0.28 log10 on treatment initiation. After 4, 8 and 12 weeks of treatment, they were 5.82±0.35, 4.05±0.55 and 2.30±0.25, respectively for RHZE; 5.00±0.09, 5.32±0.6 and 6.57±0.29, respectively for SH; and 6.00±0.39, 6.24±0.28, 7.68±0.49, respectively for RH. H resistance was prevented in RHZE treated mice but not in SH and RH treated mice.
Conclusion: Despite its bactericidal activity, S alone cannot prevent selection of H-resistant mutants in nude mice.

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Soluble guanylate cyclase stimulator riociguat prevents fibrotic tissue remodeling and improves survival in salt-sensitive Dahl rats
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Direct stimulation of soluble guanylate cyclase (sGC) is an emerging therapeutic approach to the management of cardiopulmonary disorders associated with endothelial dysfunction. Novel sGC stimulators, including riociguat, have a dual mode of action: they sensitize sGC to endogenously produced nitric oxide (NO) and also directly stimulate sGC independently of NO. Little is known about their effects on tissue remodeling and degeneration and survival in experimental malignant hypertension. Mortality, hemodynamics and biomarkers of tissue remodeling and degeneration were assessed in Dahl salt-sensitive rats maintained on a high salt diet and treated with riociguat (3 or 10 mg/kg/d) for 14 weeks. Riociguat markedly attenuated systemic hypertension, improved systolic heart function and increased survival. Histological examination of the heart and kidneys revealed that riociguat significantly ameliorated fibrotic tissue remodeling and degeneration. Correspondingly, mRNA expression of the pro-fibrotic biomarkers osteopontin (OPN), tissue inhibitor of matrix metalloproteinase-1 (TIMP-1) and plasminogen activator inhibitor-1 (PAI-1) in the myocardium and the renal cortex was attenuated by riociguat. In addition, riociguat reduced plasma and urinary levels of OPN, TIMP-1, and PAI-1. Riociguat markedly improves survival and attenuates systemic hypertension and systolic dysfunction, as well as fibrotic tissue remodeling in the myocardium and the renal cortex in a rodent model of pressure and volume overload. These findings suggest a therapeutic potential of sGC stimulators in providing organ protection in diseases associated with impaired cardio-renal functions.

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AZD3199: A fast acting β_2-receptor agonist with a long duration of action
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Background: AZD3199 is a novel, ultra long acting β_2 -agonist (uLABA) designed to combine 24 hour duration of action with a fast onset of action similar to formoterol, as well as low systemic exposure. Its *in vivo* profile was evaluated in the guinea pig.
Methods: Bronchoconstriction was elicited in anesthetized guinea pigs by histamine administration. Dose-response curves for AZD3199 given via the inhaled and intra-tracheal (i.t.) routes were constructed and sub-maximal doses used to define duration of action from 2–72 hours. The b-antagonist propranolol was administered after histamine-challenge to show the level of β_2 efficacy at each dose and time point. Blood samples were taken throughout and plasma K^+ concentrations used as a marker of systemic β_2 effects. Satellite groups were used to monitor

lung and plasma AZD3199 levels. The pharmacodynamic and pharmacokinetic profiles of AZD3199 were compared to formoterol and salmeterol.
Results: Sub-maximal doses of AZD3199 given i.t. inhibited bronchoconstriction for 24 hours; equi-effective doses of formoterol and salmeterol had significant effects for 12 hours. AZD3199 had the longest lung PK half-life. Inhalation of sub-maximal doses of nebulized AZD3199 gave bronchoprotection lasting 24 hours, with no significant effects on blood K^+ levels. An equi-effective inhaled dose of formoterol bronchoprotected for 8 hours with decreases in blood K^+ seen at 2 hours. The reduced systemic effects for AZD3199 relative to formoterol are consistent with its high lung to plasma concentration ratio.
Conclusion: AZD3199 is a novel uLABA with a fast onset of action and a longer duration of action than conventional LABAs, and also has a low potential for systemic effects.

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An assessment of the functional profile of aclidinium in human bronchi and left atria

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Introduction: Aclidinium bromide is a novel, long-acting muscarinic antagonist, currently in development for the treatment of chronic obstructive pulmonary disease.
Aims: To assess the functional profile of aclidinium in isolated human bronchi and left atria, the organs responsible for efficacy and systemic side effects, respectively.
Methods: The smooth muscle relaxant effects of aclidinium, tiotropium and ipratropium were measured in isolated human bronchial rings by determining potency, onset (time to 50% inhibition) and offset (time to 50% recovery). The effects of the muscarinic antagonists were assessed in human left-atria strips pre-treated with carbachol 10μM to inhibit electrically-induced contractions via the M_2 receptor. Duration of action was defined as the time required to recover 50% of the carbachol effect.
Results: Aclidinium had similar potency to tiotropium and ipratropium in human bronchi. Aclidinium onset (4.4±0.7 min) was faster than tiotropium (7.4±1.3 min; $p<0.05$) and similar to ipratropium (3.3±0.6 min). Aclidinium offset (334±49 min) was longer than ipratropium (76±9 min; $p<0.05$). Tiotropium did not recover within 10 h. Aclidinium inhibited the bradycardiac effect of carbachol in human left atria, with a shorter half life (110.2 min; 95% confidence interval [CI] 103.0, 117.3) than tiotropium (159.3 min; 95% CI 148.2, 171.7) but longer than ipratropium (16.6 min; 95% CI 16.4, 16.8).
Conclusions: Aclidinium has similar potency but faster onset of action than tiotropium in human bronchi. In human left atria, aclidinium had a shorter duration of action than tiotropium at M_2 receptors, suggesting a lower potential for cardiovascular side effects.