306. New drug targets and pre-clinical models for respiratory diseases

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Late asthmatic response is modulated by TRPA1 antagonists in ovalbumin-induced bronchoconstriction in anaesthetized guinea pigs

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In animal models of asthma, ovalbumin (OVA) aerosol results in bronchoconstriction characterized by a histamine-related early asthmatic response (EAR) followed by a neuropeptide-related late asthmatic response (LAR) which can be modulated by transient receptor potential (TRP) channel A1 antagonists (Thorax. 2012, 67, 19-25).

The aim of the study was to assess the potential of two TRPA1 antagonists on the EAR and LAR in OVA-induced bronchoconstriction in anaesthetized guinea pigs. Bronchoconstriction was induced by intra-tracheal administration of a single dose of OVA (50 µg/kg) and lung resistance recorded for 30 minutes. The animals were pre-treated with pyrilamine (2 mg/kg i.v.) or its vehicle (saline) 10 min before OVA. The TRPA1 antagonists HC-030031 and A-967079 or their vehicle (0.5% methylcellulose) were administered i.p. 1 h before OVA. Without pyrilamine pre-treatment, OVA induced a fast increase in lung resistance (max. 60±13 ml overflow after 1 min) which was not reduced by the TRPA1 antagonists. Under pyrilamine pre-treatment, OVA induced a slow increase in lung resistance (max. 33±10 ml overflow at the end of the recording). HC-030031 (1 µg/kg – 1 mg/kg) dose-dependently inhibited the non-histamine-related OVA- induced bronchoconstriction (ED_{50} = 0.01 mg/kg) with a maximum bronchoprotection of 76% at 0.03 mg/kg ($p<0.05$). A-967079 (1 µg/kg – 1 mg/kg) displayed the same profile as HC-030031 (ED_{50} = 0.01 mg/kg) with a maximum bronchoprotection of 78% at 0.03 mg/kg ($p<0.05$).

This study shows that the EAR is histamine related, while the LAR is modulated by the TRPA1 channel in OVA-induced bronchoconstriction in anaesthetized guinea pigs.

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Effect of nilotinib on airway smooth muscle thickening in a murine model of chronic asthma

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Background: Asthma is characterized by airway inflammation and remodeling. The tyrosine kinase inhibitor nilotinib was developed to inhibit BCR-ABL kinase activity; however, it also has potent inhibitory activity against the c-Kit and platelet-derived growth factor receptors (PDGFR). The present study aimed to determine whether nilotinib suppresses airway smooth muscle (ASM) remodeling and whether its effect is associated with c-Kit and PDGFR pathways.
Methods: We developed a mouse model of airway remodeling, which includes smooth muscle thickening, in which ovalbumin (OVA)-sensitized mice were repeatedly exposed to intranasal OVA administration twice a week for 3 months. Mice were treated with nilotinib during the OVA challenge.
Results: Mice chronically exposed to OVA developed sustained eosinophilic airway inflammation compared with control mice. In addition, the mice chronically exposed to OVA developed features of airway remodeling, including thickening of the peribronchial smooth muscle layer. Administration of nilotinib significantly inhibited eosinophilic inflammation and ASM remodeling in mice chronically exposed to OVA. Nilotinib treatment significantly reduced the expression of p-c-Kit, p-PDGFRβ, and p-ERK1/2. The expression levels of genes encoding c-Kit and PDGFRβ were also reduced by nilotinib treatment.
Conclusions: These results suggest that nilotinib administration can prevent not only airway inflammation, but also airway remodeling associated with chronic allergen challenge. Nilotinib may provide a clinically attractive therapy for chronic severe asthma.

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A comparison of anti-inflammatory compounds in a steroid-insensitive mouse tobacco smoke model

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The effects of PDE4 and p38 inhibitors were compared to steroids in a robust model of lung inflammation induced by 4 days of exposure to tobacco smoke (TS).
Methods: Mice were exposed to either air or TS for 4d and were killed 24hr after the last exposure, the lungs lavaged and cells counted. Compounds were given at maximal efficacy doses as defined in a mouse LPS model. Steroids were dosed orally (Dexamethasone, 300μg/kg 1hr pre- and 6hr post- TS); or intra-nasally (fluticasone proprionate (FP), budesonide (BUD) 300μg/kg 1hr pre- TS). Roflumilast (ROF) was dosed either i.n. (0.1, 0.3, 1mg/kg) or orally (5 mg/kg) 1hr pre-TS. The inhaled p38i PF03715444 was dosed 100μg/kg i.n 1hr pre-TS. The p38i BIRB-796 was orally (1mg/kg) dosed 1hr pre- and 6hr post-TS.
Results: TS-exposure caused cellular infiltration into the lung which was reproducible across multiple studies. Oral or i.n. dosed steroids did not inhibit the inflammation ($p>0.05$ total cell and neutrophil (neut) count). Body weight significantly decreased over the 4d (FP 6%, BUD 11%, DEX 11%; all $p<0.05$) confirming steroid availability and efficacy. ROF reduced TS-induced inflammation when given i.n. (totals -50%, neuts -60%; both $p < 0.05$) or orally (totals -50%, neuts -66%; both $p < 0.05$). The p38 inhibitors were effective dosed orally (BIRB: totals -42%, neuts -55%) or i.n. (PF: totals -50%, neuts -61%; all $p < 0.05$).
Conclusions: TS-exposure for 4d induced a steroid-insensitive lung inflammation which was reproducibly inhibited by PDE4 and P38 inhibitors; although neither caused a total inhibition of the inflammation, suggesting that there is scope to investigate more efficacious mechanisms and combinations within this model.

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Synbiotics reduce airway inflammation and improve airway function in a mouse model for COPD

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Background: Good gut health plays an important role in maintaining immunity in and beyond the gastro-intestinal tract. Respiratory disorders can be influenced by gut microbiota. Chronic obstructive pulmonary disease (COPD) is a major health problem worldwide. The disease is characterized by a progressive airflow limitation caused by an abnormal inflammatory response. Aim of this study is to investigate the effectiveness of specific dietary fibers and lactic acid producing bacteria in a mouse model for COPD.
Methods: Male BALB/c mice were instilled intra nasally (i.n.) with lipopolysaccharide (LPS, 5μg/mouse) 3x per week for 16 days. Mice were treated 5x per week by intra-gastric supplementation with: 1) Prebiotic fiber mixture of short-chain galacto-oligosaccharides and long-chain fructo-oligosaccharides (GOS/FOS), 2) Probiotic Bifidobacterium or 3) Synbiotic combination of GOS/FOS and Bifidobacterium. Broncho alveolar lavage (BAL) samples were analyzed for airway inflammation. Airway function was measured by plethysmography in anesthetized mechanically ventilated mice.
Results: LPS treatment significantly induced inflammatory cell influx in the bronchoalveolar lavage (BAL) fluid. Treatment with either GOS/FOS or GOS/FOS combined with Bifidobacterium was able to reduce the influx of macrophages and neutrophils into BAL fluid. Only treatment with synbiotics was able to attenuate the LPS induced reduction in airway function.
Conclusion: These findings suggest that a combination of Bifidobacterium and GOSFOS might be beneficial as nutritional intervention in patients suffering from COPD.
This study was performed within the framework of Dutch TI-Pharma (project number D1-101).

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Mice with immotile cilia spontaneously cough due to mechanical stimuli of postnasal drip

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Background: The underlying mechanisms of cough in patients with rhinosinusitis are poorly understood. We reported that tubulin tyrosine ligase-like family member 1 gene (*Ttll1*) knockout (KO) mice showed disorders of ciliary motility resulting in rhinosinusitis together with cough reflex.
Aim: To examine mechanisms of cough in *Ttll1*-KO mice.
Methods and results: We pathologically searched for the causes of cough and examined structural changes of the airway in KO mice. Postnasal drip (PND) was observed in the pharynx. There was no evidence of inflammation and remodeling in the lower airway. Capsaicin cough sensitivity was increased in KO mice compared to WT mice. Moguisteine, which inhibits cough via suppression of rapidly adapting receptor (RAR), decreased cough, while capsazepine, an antagonist of the transient receptor potential vanilloid subfamily 1, did not. Tosufloxacin, a fluoroquinolone antibiotic, improved nasal inflammation but accumulation of mucus and cough remained. To prove that mucus stimuli evoke cough, artificial PND were made to evoke cough in WT mice. WT mice distinctly coughed due to artificial PND. Blue dye and contrast material were administered to study nasal clearance and movement of mucus. We confirmed that nasal ciliary clearance was decreased in KO mice. Further, blocking nasal discharge from flowing to the larynx completely inhibited cough of KO mice.
Conclusions: Mechanical stimulation to larynx due to PND was transduced to the central nervous system via RAR and evoked cough in *Ttll1*-KO mice. *Ttll1*-KO mice may serve to reveal the mechanisms of cough in patients with PND and to develop new antitussive drugs.

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Intratracheal administration of dry powdered low-molecular-weight chitosan/siRNA complexes suppressed gene expression in the airway and metastatic tumors in murine lung

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Introduction: There is an increasing number of efforts to deliver small interfering RNA (siRNA) to lung. Although many different formulations with siRNA have been optimized in *in vitro* studies, only a few have been reported successful *in vivo*.
Objectives: As a carrier of siRNA, we chose low-molecular-weight chitosan (LMWC) and succeeded in producing dry powder of LMWC/siRNA complexes. In the present study, we tried to determine whether intratracheal administration of dry powdered LMWC/siRNA complexes suppressed gene expression in murine lung.
Methods: Dry powdered LMWC/siRNA targeting green fluorescent protein (GFP-siRNA) and Lewis lung carcinoma cells stably expressing GFP (LLC-GFP) were prepared. Dry powder of LMWC/GFP-siRNA complexes was intratracheally administered to GFP transgenic mice and the C57BL/6 mice injected with LLC-GFP cells through tail vein. The fluorescence in the lung tissue sections was analyzed with a BIOREVO fluorescence microscope (BZ-9000; KEYENCE, Japan).
Results: Intratracheal administration of LMWC/GFP-siRNA complexes was found to suppress the fluorescence level of bronchial epithelium in the lung of GFP transgenic mice. It was also effective at reducing the fluorescence level in metastatic lung tumors consisting of LLC-GFP cells.
Conclusion: The results of the present study suggest that LMWC is an effective carrier for siRNA delivery to the lung, and powdered LMWC/siRNA complexes may become a promising tool to knock-down a specific gene expression in lung diseases such as bronchial asthma, COPD, and metastatic lung tumors.

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ONX0912, a novel proteasome inhibitor for the treatment of lung fibrosis?

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Background: Proteasome inhibition has been shown to prevent development of fibrosis in several organs. Effects of proteasome inhibitors (PI) on lung fibrosis are controversial and cytotoxic side effects of the inhibition of proteasomal protein degradation in the cell cannot be excluded.
Hypothesis: Local administration of PI allows efficient drug delivery to the lung and prevents development of pulmonary fibrosis without systemic toxicity.
Methods and results: ONX0912 (ONX), a new irreversible PI, was evaluated in comparison to bortezomib, the only FDA approved PI, with regard to cytotoxicity and proteasomal inhibition in the cell line A549. Primary lung fibroblasts were isolated from proteasome reporter mice (ODD-luc) and characterized using MTT survival, and proteasome activity assays. The ODD-luc reporter accumulates upon proteasome inhibition and can be quantified via bioluminescence reflecting the actual degree of proteasome inhibition in the cell.
While bortezomib had strong cytotoxic effects, ONX only partially inhibited the proteasome at low doses but efficiently blocked fibroblast function without affecting cell viability of fibroblasts or epithelia cells. An optimal nontoxic dose of ONX was obtained after intratracheal instillation into ODD-luc mice. This dose then was applied locally into the lung of ODD-luc mice with bleomycin induced fibrosis and therapeutic effects were investigated by histochemical analysis of the lungs.
Conclusion: ONX provides antifibrotic effects in murine lung fibroblasts in a non-toxic dose range. Local administration of ONX into the lungs partially inhibits the proteasome without toxic side effects and can be regarded as a promising approach to inhibit pulmonary fibrosis.

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Benefits of dual bronchodilation with QVA149 once daily versus placebo, indacaterol, NVA237 and tiotropium in patients with COPD: The SHINE study

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Background: QVA149 is a novel once-daily dual bronchodilator combining the LABA indacaterol and the LAMA NVA237 (glycopyrronium) in development for COPD.
Methods: In a double-blind study, 2144 patients with moderate-to-severe COPD were randomized (2:2:2:2:1) to receive QVA149 110/50μg, indacaterol (IND) 150μg, NVA237 50μg (NVA), open-label tiotropium (TIO) 18μg or placebo (PBO), for 26 weeks. The primary endpoint was trough FEV_1 with QVA149 vs IND and NVA at 26 weeks.
Results: 89% patients completed the study. Trough FEV_1 at Week 26 was significantly greater with QVA149 vs PBO, IND, NVA and TIO (mean difference: 200, 70, 90 and 80mL, respectively; $p<0.001$). Significant improvement was also seen with QVA149 in other outcome measures evaluating lung function, dyspnea, health status and rescue medication use (table).

	Least squares mean treatment difference			
	QVA149–PBO	QVA149–IND	QVA149–NVA	QVA149–TIO
Day 1				
Trough FEV_1 (mL)[+]	190*	80*	80*	80*
FEV_1 $AUC_{0–4h}$ (mL)	220*	60*	30*	80*
Week 26				
Trough FEV_1 (mL)	200*	70*	90*	80*
FEV_1 $AUC_{0–4h}$ (mL)	340*	110*	140*	130*
FEV_1 $AUC_{0–24h}$ (mL)	320*	110*	110*	110*
Peak FEV_1 (L) (0–4h)	330*	120*	130*	130*
Transition Dyspnea Index focal score	1.09*	0.26	0.21	0.51[†]
St George's Respiratory Questionnaire total score	–3.01[‡]	–1.09	–1.18	–2.13[†]
Rescue medication use	–0.96*	–0.30[†]	–0.66*	–0.54*

[+]End of Day 1; *$p<0.001$; [‡]$p<0.01$; [†]$p<0.05$.

The incidence of adverse events was similar between groups (55% QVA149; 61% IND and NVA; 57% TIO; 58% PBO).
Conclusion: The LABA/LAMA combination of QVA149 once daily provided significantly superior, rapid and sustained bronchodilation vs PBO, IND, NVA and TIO, with significant symptomatic improvements and a safety profile similar to PBO.