484. Translational models of airway disease

4683
Bitter taste receptor agonists as a novel class of bronchodilators in guinea-pig airways
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Rationale: Deshpande et al. (Nat Med 2010) reported that several bitter taste receptor (TAS2R) agonists evoked relaxation of mice and human airways. We examined the effects of three prototype agonists in segments of guinea pig trachea (GPT).

Methods: GPT was pre-contracted with 0.1 μM carbachol in both absence and presence of 3 μM indomethacin or prostaglandin antagonists. The segments were either exposed to denatonium, chloroquine or saccharin, or kept untreated. Expression of TAS2Rs in guinea pig tracheal epithelium and smooth muscle was measured with real-time PCR.

Results: Denatonium and chloroquine induced concentration-dependent relaxations whereas saccharin had no effect. In consistency with these findings, there was expression of TAS2R4 and TAS2R10 for denatonium, and TAS2R3 and TAS2R10 for chloroquine, but not of TAS2Rs for saccharin in guinea pig airways. Denatonium was 6.1-fold more potent than chloroquine (pEC50 4.7±0.1 and 3.8±0.1, respectively). Indomethacin had no effects on the potency of denatonium and chloroquine. However, the magnitude of the denatonium-induced relaxation (57.5±5.2%; n=8) was enhanced by indomethacin (97.7±2.3%; n=8) and the prostaglandin E2 receptor (EP1) antagonist ONO-8310 (99.3±0.7; n=5). Chloroquine induced almost complete relaxation (98.2±1.1%; n=6) that was unaffected by indomethacin (99.9±0.04%; n=7).

Conclusion: Denatonium and chloroquine induced relaxation of GPT and their respective TAS2Rs were expressed. There was an interaction between denatonium and PGE2 acting on EP1 receptors. The findings support the concept that airway TAS2Rs represent a novel target for anti-asthmatic therapy.

4684
Prostacyclin modifies VEGF synthesis in fibroblasts from healthy and COPD patients
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Background: Involvement of vascular remodelling in the lung is a characteristic sign in chronic obstructive lung disease (COPD). The vascular mediator prostacyclin may regulate fibroblast activity. The objective was to study the effect of prostacyclin on synthesis of vascular endothelial growth factor (VEGF) and interactions with transforming growth factor (TGF) B1 in distal lung fibroblasts from patients with COPD and control subjects.

Method: Primary human lung fibroblast cultures were established from peripheral airway biopsy samples from healthy individuals (controls, n=5) and after lung resection from patients with COPD (GOLD IV) (n=7). The lung fibroblasts were cultured in 0.4% medium and stimulated with the cyclooxygenase inhibitor indomethacin 3μM in combination with the prostacyclin analogue iloprost 1μM and TGF-B1 10ng/ml for 24h. VEGF production was measured in the cell culture supernatant by ELISA.
Conclusions: Iloprost enhanced VEGF synthesis in both fibroblasts from control subjects (p = 0.05) and 6.1-fold from patients with COPD (p = 0.01). However, iloprost showed no effect on VEGF synthesis after TGF-β stimulation, whereas indomethacin reduced Iloprost production in fibroblasts from patients with COPD (p < 0.05) but not in control subjects.

4687

Protective effect of a protein epitope mimetic (PEM) CCR10 antagonist, POL7085, in an allergic model of asthma

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Potential involvement of the CCR10/CCL28 axis was recently reported in a murine model of allergic asthma [1]. Blockade of the CCR10 receptor might therefore represent a novel alternative to the current treatments of asthma. We have screened for the effect of the PEM CCR10 antagonist, POL7085, in an allergic model of asthma in mice. Nine week-old male Balb/c mice were sensitized to ovalbumin (OVA) administered intraepidermically in the presence of alum (D0 and D7), and challenged to OVA administered intranasally (D18 to D21). POL7085 was administered once daily 1 hour before each OVA challenge at 9 and 18nmol/kg intra-nasally (I.N.) vs dexamethasone I.N. (DEX, 2.5nmol/kg) vs vehicle. In treated animals, OVA induced airway hyperresponsiveness (AHR) as measured by whole body plethysmography, and hypereosinophilia in the bronchoalveolar lavage (BAL) fluid. POL7085 dose-dependently and significantly decreased AHR by 34±16% and eosinophil numbers in BAL by 66±6%. In addition, the higher dose of POL7085 also inhibited IL-5 secretion in BAL (24±13%, IgE and IgG1 synthesis in serum (47±3% and 61±15%, respectively), and lung collagen synthesis (43±11%), although not significantly. POL7085 as compared to DEX also modified body (6.5±1.7% vs 4.5±1.5% for DEX) and spleen weight (24±3% vs 44±3% for DEX).

In conclusion, the PEM CCR10 antagonist, POL7085, significantly and dose-dependently decreased asthma symptoms after once daily local administration in particular AHR and eosinophilia. Blocking the CCR10 chemokine receptor therefore appears as a promising novel approach for treating asthma.


4688

Characterisation of TNF-α-lectin-like domain derived peptides associated with improved alveolar fluid clearance in pulmonary oedema

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The beneficial effect of the lectin-like domain of TNF-α, including the TIP peptide which mimics this domain, on activation of oedema resorption, improved alveolar clearance and protection of lung function after transplantation, is well documented from several independent in vitro and in vivo studies using animal models. The effect is mediated by activation of sodium uptake from the alveolar fluid through ENaC and improving clinical outcome in this condition.

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4689

Consequences of chronic pulmonary TLR9 activation in the lung and beyond

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Background: Toll like receptor 9 (TLR9) agonist Cpg-G129 is being explored as an anti-allergic drug for asthma. However TLR9 could play a role in COPD. Cigarette smoke induced-IL-8 production is partly TLR9 mediated.

Aim: To investigate the (extra)-pulmonary effects of Cpg-G129. We hypothesized that pulmonary TLR9 activation induces neutrophil influx which could lead to adverse effects.

Methods: A single dose of 0.01, 0.05 or 0.25mg/Ml/gBW Cpg-G129 was targeted to balbc mice lungs by aspiration (acute). Next 0.01mg/gBW Cpg-G129 was administered repeatedly for 5 days (chronic) or 5 weeks (chronic). 24 hours after last exposures, measurements were done: lung function; hypertension and abstract printing supported by G Chiesi. Visit Chiesi at Stand D.30
485. Smoking-related disorders

4691 Late-breaking abstract: Effectiveness of easy smoking cessation clinic in tertiary health care settings: Observational study of cohorts
Ratapun Champunot, Maneewan Waikasikorn, Pranomm Maweha, Aina Chotopong, Phanpạn Pongsathorn, Siriwan Awasthit, Anincha Pongphit, Mataneeya Pohkhor, Sirawat Sawanakitt, Sunee Jirasmit, Internal Medicine, Buchachainnaraj Hospital, Phitsanulok, Thailand

Introduction: Tobacco treatment programs should be offered in clinical settings for all smokers who need to quit smoking. We have co-operated with every units and departments in hospital and changed our service patterns of smoking cessation clinic into easy way. Cessation assistance was provided on working time every day except holiday. It requires approximately 20-30 minutes to complete and involves asking patients about smoking behavior and acting to help them quit. Telephone helpline was used to follow up and encourage smokers trying to continue quit smoking.

Methods: This is an observational study of cohorts of participants in smoking cessation clinic, Buchachainnaraj hospital during June 1 to November 30, 2009. The main outcome measurements were self report abstinence rate at 6 and 12 months, and cost per quit.

Results: Over a period of 6 months, a cohort of 315 smokers were enrolled in this study. The self report abstinence rate at 6 and 12 months was 33.7% (106/315) and 27.9% (88/315) The mean cost per quit was 3.145 baht (70 Euro).Lost follow up rate by telephone helpline at 6 and 12 months was 18.4% and 27.6%.

Conclusions: Easy smoking cessation clinic is the intervention that are simple, cheap, and effective Strategies for incorporating effective smoking cessation clinic into routine clinic care needs to become a key part of routine intervention for managing smoking cessation.

4693 Cannabis use in patients with a primary spontaneous pneumothorax
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Introduction: It’s Dutch policy to tolerate cannabis use. In literature active cannabis use is 10% amongst Dutch youth. The association between cannabis use and primary spontaneous pneumothorax (PSP) is unknown.

Aim: To determine the frequency of cannabis use in addition to tobacco smoking in patients with a PSP and to investigate the presence of underlying abnormalities on High Resolution CT (HRCT).

Methods: In a descriptive retrospective study patients were included who pre-sented in a large Dutch teaching hospital with a PSP between august 2008 and august 2010. Because of an increased risk on secondary pneumothorax in older patients, only patients under 50 years were included. Age, gender, BMI, tobacco (T) and cannabis (C) use and when available HRCT data were recorded.

Results: In 2 years 53 patients presented with a PSP (42 male, 11 female, mean age 28 years, mean BMI 21). 74% (8% ex) smoked tobacco, 49% (8% ex) used cannabis (cannabis use unknown in 6%). The findings on HRCT are presented in Table 1.

Table 1. HRCT findings

<table>
<thead>
<tr>
<th>(N (%))</th>
<th>bulb &amp;</th>
<th>normal HRCT</th>
<th>unknown</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>30 (57)</td>
<td>11 (19)</td>
<td>13 (24)</td>
</tr>
<tr>
<td>C&amp;T+</td>
<td>(no C without T) 15 (56)</td>
<td>2 (3)</td>
<td>13 (28)</td>
</tr>
<tr>
<td>C–T–</td>
<td>7 (23)</td>
<td>5 (36)</td>
<td>3 (21)</td>
</tr>
<tr>
<td>C–T+</td>
<td>6 (43)</td>
<td>1 (7)</td>
<td>3 (21)</td>
</tr>
</tbody>
</table>

Conclusions: In patients under 50 years with PSP the use of cannabis was much higher than in the general population. However, all cannabis users also smoked tobacco. Only 12% of the cannabis users had a normal HRCT (30%). On HRCT, bulbare were present in 87% of cannabis users, in contrast to 57% in only tobacco smokers and none in nonsmokers.

4694 Cigarette smoke induces β2-integrin-dependent neutrophil migration across human umbilical vein endothelium
Aaskia Overbeek, Saskia Braber, Paul Hendricks, Marjke Kleinjan, Vera Kamp, Niki Georgiou, Johan Garssen, Aletta Kranevelde, Gert Folkerts. Division of Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Faculty of Science, Utrecht University, Utrecht, Netherlands. Department of Respiratory Medicine, Utrecht Medical Center Utrecht, Utrecht, Netherlands Danone Research, Danone Research Centre for Specialised Nutrition, Wageningen, Netherlands

Background: Cigarette smoke induces peripheral inflammatory responses in all smokers and is the major risk factor for neutrophilic lung diseases such as chronic obstructive pulmonary disease. The aim of this study was to investigate the effect of cigarette smoke on neutrophil chemotaxis and on β2-integrin activation and function in neutrophilic transmigration through endothelium.

We assumed that even in asymptomatic young smokers, with relatively short smoking duration and normal lung function, induced sputum could be found some changes indicative for early inflammatory process. Aim: The aim of this study was to evaluate morpho-functional changes in airways of young cigarette smokers.

Method: We enrolled 236±3 years old 12 non-alcoholic smokers (1.5±0.67 pack-years) and 7 healthy non-smoking volunteers. Lung function measurements, sputum induction (IS) and sputum cell analysis were performed. Results: Demographic data for both study groups did not differ significantly. Non-smokers and smokers had normal lung function indices. In smokers induced sputum contained statistically significantly (p=0.026) increased relative count of eosinophils 0.923 (0.355-1.753)% compared with non-smokers 0.069 (0.046 - 0.550%). We also found significant reduction of absolute (n=0.482; p=0.037) and relative (n=0.68; p=0.004) count of bronchial epithelial cells in induced sputum that correlated to number of smoked pack-years. A trend towards statistical significance showed the correlation between smoked pack-years and the relative number of macrophages in induced sputum (r=0.42; p=0.087). A trend towards statistical significance was also found in correlation between smoked pack-years and diminished FEV1% of predicted (r = -0.463; p=0.046).

Conclusions: In this study we showed that even smokers with short duration of the smoking habit have already initial signs of inflammation with eosinophil involvement.
Methods and results: Utilizing freshly isolated human neutrophils, the effect of cigarette smoke on chemotaxis and β2-integrin activation was studied. We demonstrate that cigarette smoke extract (CSE) dose dependently induced chemotaxis of neutrophils in vitro. Moreover, CSE promoted neutrophil adherence to fibrinogen. Using functional blocking antibodies against CD11b and CD18, it was shown that Mac-1 (CD11b/CD18) was responsible for the cigarette smoke-induced firm adhesion of neutrophils to fibrinogen. Furthermore, neutrophils transmigrated through endothelium by cigarette smoke due to the activation of β2-integrins, since pre-incubation of neutrophils with functional blocking antibodies against CD11b and CD18 attenuated this transmigration.

Conclusion: This is the first study to describe that cigarette smoke extract is a direct chemo-attractant for neutrophils and an activator of β2-integrins on the cell surface. Blocking this activation of β2-integrins might be an important target in cigarette smoke induced neutrophilic diseases.

4695 Waterpipe smoking and dependency are associated with chronic obstructive pulmonary disease: A case control study
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Waterpipe (WP) smoking is gaining in popularity in the Lebanese population. Although WP smokers are potentially exposed to the same noxious substances found in cigarettes, popular belief considers WP smoking harmless. Our objective was to evaluate the association between WP smoking, dependence and COPD.

We conducted a case-control study in two tertiary-care hospitals. Cases were included if diagnosed as COPD by a chest physician and confirmed by a postbronchodilator spirometry (FEV1/FVC<0.7); controls included were free of any respiratory disease or symptom. After an oral informed consent, a standardized questionnaire was administered and spirometry results were collected by trained technicians.

211 COPD cases and 554 healthy controls were enrolled. In previous smokers, any type of smoke was associated with COPD: OR=28.3 (p<0.001) for cigarette smoking, OR=12.2 (p<0.001) for water pipe smoking, and OR=41.9 (p<0.001) for mixed smoking. Lower odds ratios were found in current smokers: OR=19.6 (p<0.001) for cigarette smoking, OR=4.8 (p=0.299) for waterpipe smoking and OR=9.5 (p<0.001) for mixed smoking. However, assessing WP dependence by the validated LWD5-11 scale in current WP smokers, found an OR=15.0 (p=0.001) for the association between WP dependence and COPD. These results were confirmed by stratified and multivariate analysis, after adjustment for cigarette smoking and other potential confounding variables.

This is the first study that looked at the relation between COPD and WP smoking, and that WP smoking is a risk of COPD in current smokers of WP. In current smokers of WP, dependent individuals have an increasing risk of COPD, as much as cigarette smokers.

4696 The mother’s smoking during pregnancy influences on endothelial dysfunction in newborns
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Smoking during pregnancy is a risk for dangerous consequences. Smoking causes increased chances for miscarriage, growth restriction, preterm birth, and health problems in the future.

The aim of the investigation was to reveal effect of mother’s tobacco smoking during pregnancy on endothelial dysfunction in newborn. 127 children born from mothers - smokers were examined. The control group was consisted of 32 healthy babies born from never smoking parents. A level of thiocyanate (a marker of passive smoking) was determined in a blood serum by spectrophotometry method. Levels of S-nitrosothiols and endotheline-1 were determined in blood serum by spectrophotometry and enzyme immunoassay methods respectively for the estimation of the endothelium dysfunction.

The levels of thiocyanate were 8,64±0,11fmol/ml in I group, 2,37±0,16fmol/ml in II group (P1<0,001; P2<0,001); 1,29±0,11fmol/ml in II group (P2<0,001) for cigarette smoking, OR=12.2 (p<0.001) for waterpipe smoking, and OR=41.9 (p<0.001) for mixed smoking. Lower odds ratios were found in current smokers: OR=19.6 (p<0.001) for cigarette smoking, OR=4.8 (p=0.299) for waterpipe smoking and OR=9.5 (p<0.001) for mixed smoking. However, assessing WP dependence by the validated LWD5-11 scale in current WP smokers, found an OR=15.0 (p=0.001) for the association between WP dependence and COPD.

2-integrins on the cell surface. Blocking this activation of 2-integrins, since pre-incubation of neutrophils with functional blocking antibodies against CD11b and CD18 attenuated this transmigration.

Conclusion: This is the first study to describe that cigarette smoke extract is a direct chemo-attractant for neutrophils and an activator of β2-integrins on the cell surface. Blocking this activation of β2-integrins might be an important target in cigarette smoke induced neutrophilic diseases.