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The bronchodilator effects of the novel anti-inflammatory compound TPI 1020, alone and in combination with formoterol, in conscious guinea pigs
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TPI 1020 is a novel anti-inflammatory compound with a dual mechanism of action involving corticosteroid activity and nitric oxide (NO) donation. It is hypothesised that the NO released may exert a bronchodilator effect. This study examined the individual and combined bronchodilator effects of TPI 1020 and the long-acting β_2 -adrenoceptor agonist, formoterol, in conscious guinea-pigs.

Male, Dunkin-Hartley guinea-pigs (350-400g) were exposed to a 20sec, nose-only, 3mM histamine challenge. Whole body plethysmography was used to measure specific airway conductance (sG_{aw}) immediately and at 5 and 10min post-challenge. 24hrs later, animals received nebulized formoterol (0.3-10 μ g/ml), TPI 1020 (0.11-0.7mM), combined TPI 1020 and formoterol, or vehicles for TPI 1020 (30%DMSO:30%EtOH:40% saline) or formoterol (saline) for 15min. The histamine challenge was repeated 15 or 75min later.

TPI 1020 (0.3mM) significantly inhibited histamine-induced bronchoconstriction by 50.5 \pm 8.1% ($p < 0.01$) 15mins post-administration but had no activity at 75mins. Formoterol inhibited dose-dependently over the dose range (0.3-3 μ g/ml), up to 70.8 \pm 16.6% of the control histamine response at 75mins. Co-administering 1 μ g/ml formoterol, which alone had a small non-significant inhibitory effect (24.9 \pm 7.9%) with TPI 1020 (0.11 or 0.33mM) significantly ($p < 0.05$) inhibited the histamine response by 45.7 \pm 12.2% and 63.8 \pm 14.2%, respectively, 75min post-administration.

Co-administering formoterol with TPI 1020 has the potential to elicit a more potent bronchodilator effect. Such a combination could therefore be of further interest for treatment of bronchoconstriction-associated disease.

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Effect of Mn(III) tetrakis (4-benzoic acid) porphyrin on inflammation and fibrosis in amiodarone-induced pneumotoxicity in rats
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New catalytic antioxidants metalloporphyrins have been proved to have protective role in inflammatory conditions, inhibiting inflammatory gene expression in response to reduced generation of reactive oxygen species such as superoxide, peroxide, peroxyxynitrite and lipid peroxy radicals. We examined the effect of manganese (III) tetrakis (4-benzoic acid) porphyrin (MnTBAP) on amiodarone (AD)-induced pulmonary toxicity in the rat model.

Methods: The study was carried out on 48 male Wistar rats, divided into four groups: (1) – controls; (2) – treated intratracheally (i.t.) with AD; (3) – treated with AD and MnTBAP; (4) – treated with MnTBAP. AD was administered i. t. on days 0 and 2 (6.25 mg/kg). MnTBAP was injected intraperitoneally at a dose 10 mg/kg on day 0, 1 and 2. Cytologic and biochemical (activity of lactate dehydrogenase (LDH), acid phosphatase (AcPh), alkaline phosphatase (AlPh) assays of bronchoalveolar lavage fluid (BALF) was performed on day 3. Pulmonary fibrosis was assessed by measuring hydroxyproline (HP) content in lung homogenate (LH) on day 28 after AD administration.

Results: AD treatment resulted in significantly increased protein content; total cell count; polymorphonuclear cells; activity of LDH, AcPh and AlPh; and content of

HP. The treatment with AD and MnTBAP decreased the markers of pulmonary inflammation and cytotoxicity in BALF compared to AD group. The content of HP in AD + MnTBAP (2.25 \pm 0.16 mcg/ml LH) group was decreased compared to AD alone (3.34 \pm 0.15 mcg/ml LH) on day 28 ($p < 0.05$).

MnTBAP reduced early AD-induced lung inflammatory injury and can protect animals from AD-induced pulmonary fibrosis.

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Effects of AT1 receptor blockade on pulmonary and systemic manifestations in a COPD/emphysema mouse model

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Background: We recently developed a COPD/emphysema mouse model exhibiting systemic manifestations of the disease¹. Aim was to investigate the effects of AT1 receptor blockade on pulmonary and systemic manifestations in this animal model.

Methods: Female NMRI mice were treated 5 \times intratracheally with porcine pancreatic elastase (em, n=11) or with phosphate-buffered saline (co, n=4). Afterwards 6 of the em animals received the AT1 blocker irbesartan (em-irb) orally (50 mg/kg BW) for 8 weeks. Evaluated outcome parameters included exercise tolerance (treadmill running distance), neurohumoral activation (urinary norepinephrine), and lung emphysema (mean alveolar intercept and static compliance).

Results: Mean linear intercept was 35.0 \pm 0.6 μ m in co, 121.1 \pm 6.6 μ m in em (p vs co < 0.001) and 88.6 \pm 3.2 μ m in em-irb (p vs em < 0.001). Static compliance was significantly higher in em compared to em-irb and co (354.4 \pm 61.2 μ l/cm H₂O vs 163.3 \pm 48.4 μ l/cm H₂O vs 46.6 \pm 8.4 μ l/cm H₂O). The relative running distance 5 days after elastase treatments was significantly shorter in both em groups compared to baseline. Em-irb animals recovered and showed a significantly higher relative running distance after 8 weeks of treatment ($p < 0.01$) in contrast to em animals without irb. Norepinephrine in urine was significantly lower in em-irb than in em (19.1 \pm 3.7 μ g/l mg/100ml creatinine vs 26.9 \pm 3.0 μ g/l mg/100ml creatinine; $p = 0.02$).

Conclusion: This study indicates a favourable effect of AT1 receptor blockade on systemic manifestations and structural lung changes in a COPD/emphysema model with neurohumoral activation. Underlying mechanisms need to be further evaluated.

1. Luthje L, et al. Respiratory Research 2009;10:7.

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Acrolein induces airway remodeling in mice via NF- κ B pathway

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Background: Acrolein, an unsaturated aldehyde found in smog and tobacco smoke, can induce airway hyperreactivity, inflammation, and mucus hypersecretion, which are features of COPD. However, the role of acrolein in airway remodeling remains unclear.

Objectives: To investigate whether acrolein could induce airway remodeling and the effect of caffeic acid phenethyl ester (CAPE) in this process.

Methods: C57BL/6 mice were exposed to acrolein (4.0 ppm; 6 h/day) for 6 wk and administered with CAPE at dose of 30 mg/kg qod. Lung tissue sections were stained with Masson's Trichrome for morphological analysis. Hydroxyproline content in trachea was measured. The expressions of TGF- β 1 and NF- κ B were detected by RT-PCR, ELISA, immunohistochemistry and western blotting.

Results: Acrolein exposure resulted in augmented Masson-stained area in airway wall and the increased expression of TGF- β 1 in lung. CAPE administration diminished Masson-stained area in airway wall, moreover, CAPE significantly decreased TGF- β 1 protein level in BAL fluid and TGF- β 1 mRNA expression in lung. The increases of NF- κ B immunostaining in mouse airway epithelium and NF- κ B protein expression in lung tissue that followed acrolein exposure were also suppressed by CAPE administration.

Conclusions: Our findings suggest acrolein exposure induces airway remodeling in vivo, which can be significantly attenuated by CAPE treatment via inhibition of NF- κ B pathway.

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Inhibition of c-kit tyrosine kinase by sunitinib alleviates airway remodeling in a mouse model of chronic asthma

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Objectives: To investigate the effects of sunitinib, a tyrosine kinase inhibitor, on airway remodeling and to explore its application prospect for treatment of asthma.

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Methods: Mice were divided into 5 groups with 10 mice in each. They were asthma group, sunitinib group, budesonide group, and combined treatment group, and normal control group. In the first 4 groups mice were immunized and challenged with ovalbumin to establish the chronic asthmatic model. Histological changes were observed by the means of HE stain, Masson Trichrome stain, PAS stain and immunohistochemistry. IgE and IL-13 levels were determined by ELISAs. Expressions of phosphorylated pKIT were determined by Immunoprecipitation/Western blot analysis.

Results: Compared with other groups, the mice in asthma group were significantly more sensitive to methacholine and revealed markedly development of airway remodeling with significantly elevated levels of IgE and IL-13. However, in sunitinib, budesonide or combined treatment groups the above-mentioned changes were significantly weakened.

Conclusions: The data demonstrated that sunitinib could alleviate airway inflammation and inhibit airway remodeling in the mouse model of chronic asthma.

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Bronchoprotective role of PGE₂ synthesized via microsomal PGE synthase on airway hyperresponsiveness *in vivo*

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Previous observations suggest that cyclooxygenase products modify airway inflammation and hyperresponsiveness (AHR) following OVA-challenge in mice. Microsomal PGE synthase (mPGES) is believed to catalyze the formation of PGE₂ in inflammation. In this study we investigated the role of PGE₂ generated by mPGES in a mouse model of chronic allergic airway inflammation.

C57Bl/6 wt or mPGES knock out (KO) mice (n=9-12), were sensitized to ovalbumin (OVA; 10µg in alum) i.p. on day 1 and 11 and challenged with 100µg OVA intranasally on two consecutive days every second week for 12 weeks. AHR was measured based on total lung resistance (R_L) to i.v. injection of methacholine (MCh; Flexivent®, Scireq) 24h after the last OVA challenge, followed by collection of BAL fluid for total cell count.

In OVA challenged males, maximal R_L was increased in wt compared to PBS challenged controls (3.9±0.4 and 2.3±0.1cmH₂O·s/ml respectively, p<0.05). Interestingly, mPGES KO had a further enhanced R_L compared to wt (6.0±1.2 and 3.9±0.4cmH₂O·s/ml respectively p<0.05). Similar to male KO mice, maximal R_L was increased in female wt compared to PBS challenged controls (3.0±0.4 and 1.5±0.07cmH₂O·s/ml respectively, p<0.05). This increase was further enhanced in mPGES KO compared to wt following OVA challenge (3.0±0.4 and 2.0±0.2cmH₂O·s/ml respectively, p<0.05). The total BAL cell number was augmented after OVA challenge in both wt and mPGES KO mice, in both males and females but there were no differences between wt and KO.

The findings indicate that PGE₂ generated via mPGES has a bronchoprotective function and reduces AHR during allergen challenge without affecting infiltration of cells into the airways.

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Anti-asthmatic effects of losartan, a blocker of angiotensin II type 1 receptor, in a rat model of allergic asthma

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Literature data demonstrate the angiotensin II (Ang II) involvement on airway hyperreactivity and inflammation, recognizing its role in allergic asthma pathophysiology. To verify if blocking of pulmonary (local) renin angiotensin system (RAS) could have beneficial effects on development of allergic lung diseases. The experiments were conducted in three groups of male Wistar rats: normal (NR), ovalbumin sensitized (OSR) and ovalbumin sensitized but losartan (a blocker of angiotensin II type 1 receptor) treated (OSR-L). The losartan (solution 5mM) was administered by nebulization each day on the last week of sensitization protocol. The OVA-induced bronchoconstriction was reduced by more than 40% on OSR-L as compared with OSR. The acetylcholine (ACh) – induced contractions were significantly higher on OSR than on NR but losartan treatment reduced the amplification of Emax of ACh with more than 20%. On OSR-L the terbutaline (cumulative doses, 1 nM – 1 mM) induced relaxation of ACh precontracted bronchial rings is significantly powerfully (Emax is increased by 1.5 times and the logEC50[M] is decreased with 0.42) than on OSR. There was no significant difference between NR and OSR-L on the total number of cells from bronchoalveolar lavage fluid. Histological examination revealed that losartan treatment strongly decreased infiltration of inflammatory cells both on the walls of lobar and segmental bronchi and in peribronchiolar/ perivascular space. These results suggest that inhalatory administration of losartan reduces effects of OVA-challenge in sensitized rats and indicates pulmonary RAS blockade as a potential antiasthmatic tool.

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Inhaled CCR1 antagonist AZD4818 reduces leucocyte influx in the rat lipopolysaccharide (LPS) lung inflammation model

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Background: The chemokine receptor CCR1 is expressed on leukocytes and belongs to the seven-transmembrane G-protein-coupled receptor (GPCR) superfamily of receptors. CCR1 antagonists reduce cell infiltration and disease development in rodent models of rheumatoid arthritis, multiple sclerosis, transplantation, kidney disease and asthma.

Methods: The LPS acute 4-hour lung inflammation rat model was used. To achieve inhaled administration of the specific small molecule CCR1 antagonist AZD4818, it was dissolved in the vehicle and then vaporised with the resulting aerosol droplets being actively inhaled for 10 min. AZD4818 was given 30 min prior to intratracheal LPS challenge; 4 hours after LPS administration bronchoalveolar lavage (BAL) samples were taken.

Results: Nebulised AZD4818, in estimated lung deposited doses 0.3–26 µg/kg body weight (BW), significantly and dose dependently reduced leucocyte infiltration, especially the neutrophil accumulation, in rat BAL (0.3 µg/kg BW (0.577 nmol/kg): 65% reduction; 1 µg/kg BW (1.92 nmol/kg): 75% reduction; 26 µg/kg BW (50 nmol/kg): 87% reduction of BAL neutrophil influx).

Conclusions: These pharmacological findings indicate that the inhaled CCR1 antagonist AZD4818 displayed remarkable treatment potency in the rat LPS model and could be effective in reducing inflammatory cell influx and potentially symptoms in human respiratory disease.

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