223. Models of disease and drug actions

P1773

Inhaled budesonide induces corticosteroid-dependent gene expression in asthmatics: Validation in primary epithelial and airways smooth muscle cells

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Rationale: Inhaled corticosteroids (ICS) reduce inflammatory gene expression. This is usually attributed to direct inhibition of inflammatory gene transcription by the glucocorticoid receptor. However, while corticosteroids induce anti-inflammatory gene expression *in vitro*, this has not been examined in asthmatic subjects taking ICS.
Methods: Bronchial biopsies from atopic asthmatics taking inhaled budesonide (2×200 μg, twice daily for 11 days) or placebo were subjected to gene expression analysis using real-time reverse transcriptase-polymerase chain reaction. mRNA expression for the corticosteroid-inducible genes; TSC22D3 (GILZ), DUSP1 (MKP-1), both anti-inflammatory effectors, and FKBP5 (FKBP51), a regulator of glucocorticoid receptor function, was assessed. Cultured pulmonary epithelial and smooth muscle cells were also treated with corticosteroids before gene expression analysis.
Results: Expression of GILZ and FKBP51 were significantly elevated in budesonide-treated subjects compared to placebo. Budesonide also increased GILZ expression in cultured epithelial and smooth muscle cells and immunostaining showed GILZ expression in the airways epithelium and smooth muscle of asthmatic subjects.
Conclusions: Expression of corticosteroid-induced genes, including the anti-inflammatory gene, GILZ, is upregulated in the airways of asthmatic subjects taking medium daily doses of inhaled budesonide. The biological effects of such genes need to be considered when assessing ICS action.
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P1774

Change in dilation effect of prednisone at the different stages of COPD

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Corticosteroids are widely used in treatment of COPD. However there is no consensus about their effectiveness at different stages of COPD.
Aim: To evaluate the effect of prednisone (P) on smooth muscle (SM) contraction of bronchi isolated from rats with different stages of COPD.
Methods: Model of COPD was induced in Wistar rats by long-time nitrogen dioxide (NO2) exposure (15 ppm, 1,5 h/day, 60-90 days). Model adequacy was confirmed morphologically and immunohistochemicaly. The bronchial SM contractility was evaluated at different stages of COPD (15, 30, 60, 90 days NO2 exposure). Bronchi (2-6 generations) with intramural ganglions were isolated and placed in perfusion solution. P (10 μg/ml) was added in perfusion solution. SM contractions were determined at electric stimulations preganglionic nerves or SM using the electromechanical displacement sensor.
Results: After 15-day exposure NO2 (acute stage) P decreased the amplitude of bronchial SM contraction caused by stimulation of preganglionic nerves to 29,4±2,5% of the initial level ($p<0,01$), after 30-day exposure – to 14,7±3,8% ($p<0,05$), at 60- and 90-day exposure effect of P was absent. With SM stimulation the application of P didn't affect on SM contraction for any duration NO2 exposure. After 90 days in response to SM stimulation prednisone instead of reducing the SM contraction caused its increase up to 111,0±4,9% ($p<0,05$).
Conclusion: Bronchodilatory effect of P is mediated by neurogenic mechanism. The greatest effect of P manifests in the initial stages of formation of COPD. As the progression of COPD the dilator effect of P is reduced until disappearance (corticosteroid resistance).

P1775

Inhibition of glycogen synthase kinase 3beta reduces GC function in human monocytes via modulation of HDAC2 activity

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Chronic obstructive pulmonary disease (COPD) is a progressive inflammatory lung disease characterized by insensitivity to glucocorticoids (GCs). Reactive oxygen species (ROS) are an etiologic factor in the pathogenesis of COPD and contribute to the reduced GC action. Insensitivity to GCs has been associated with impaired catalytic activity of HDAC2 in the lungs of COPD patients. GSK3beta is a constitutively active kinase that regulates cell cycle and gene expression. Phosphorylation of Ser9 on GSK3beta leads to its inactivation and elevated levels of the phospho-GSK3beta have been documented in the peripheral lung of COPD patients.
Monocytes from healthy volunteers were treated with H2O2 which induced GSK3beta Ser9 phosphorylation in a concentration- and time-dependant manner. Use of selective inhibitors showed that the ERK pathway mediates the ROS induced GSK3beta phosphorylation. To examine how inactivation of GSK3beta regulates GC function, we assessed the effect of a selective inhibitor (CT99021) on GC-mediated inhibition of GM-CSF release. The data showed that inhibition of GSK3beta alone had no significant effect on LPS induced GM-CSF release but it attenuated the ability of dexamethasone to suppress GM-CSF release by up to 50%. Selective inhibition of GSK3beta also significantly decreased HDAC2 activity suggesting that HDAC2 is an important mediator in this pathway.
Our results indicate that ROS induces inhibition of GSK3beta via the ERK pathway leading to decreased HDAC2 activity and GC insensitivity. This pathway should be considered in evaluating the therapeutic potentials of GSK3beta activators in restoring GC sensitivity in COPD.

P1776

Effects of beclomethasone dipropionate and formoterol in reducing oxidative stress induced by cigarette smoke extracts and IL-17

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Rationale: Oxidative stress is involved in airway inflammatory diseases. Inhaled-corticosteroids reduce airway inflammation and the combination with long-acting β2 agonists enhances this effect.
Objective: To investigate whether Cigarette smoke extracts (CSE) and Interleukin-17A (IL-17A) activate airway epithelial cells to release markers of oxidative/nitrosative stress and to investigate the effect of beclomethasone dipropionate (BDP) and formoterol.
Methods: Human bronchial epithelial cells (16HBE) were stimulated with different concentrations of CSE (from 0 to 10%) to evaluate the expression of IL-17 receptor (IL-17R). 16HBE were also stimulated with CSE (2.5%) with and withouth rhIL-17A (50 ng/ml) to evaluate the production of Reactive oxygen species (Ros), and Nitrotyrosine levels. The effects of BDP (10^{-8}M) and Formoterol (10^{-8}M), alone and in combination, were evaluated.
Results: CSE increased the expression of IL-17R in 16HBE in a dose dependent manner with a maximum effect at 2.5% concentration ($p<0.001$).
Both CSE and rhIL-17A separately increased the production of Ros and Nitrotyrosine ($p<0.05$) and their combination synergistically further increased the production of these markers ($p<0.001$). BDP alone was able to completely restore the baseline values in terms of IL-17R expression ($p<0.001$) and its combination with Formoterol was superior in reducing the Ros and Nitrotyrosine production ($p<0.001$).
Conclusions: Cigarette smoke and IL-17A increase the production of oxidative/nitrosative markers in human bronchial epithelial cells, this effect being reduced by BDP either alone or combined with Formoterol.
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P1777

Activated protein phosphatase PP2A by formoterol enhances nuclear translocation of glucocorticoid receptor induced by budesonide

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Introduction: We have reported that formoterol (FM), a long-acting β_2- adrenoceptor agonist, restores corticosteroid (CS) sensitivity by activation of a serine/threonine protein phosphatase PP2A (ERJ 2009;34:583s). However, the molecular mechanisms how FM activates PP2A and restores CS sensitivity have not been elucidated.
Aims: To investigate the mechanism of PP2A activation by FM and the involvement of PP2A in glucocorticoid receptor (GR) nuclear translocation induced by CS budesonide (BUD).
Methods: Phosphatase activity of immunopurified PP2A from U937 monocytic cells was measured by fluorescence-based assay. A549 lung epithelial cells, without functional β_2-adrenoceptor, were used as control cells. Direct effect of FM was evaluated using PP2A immunopurified from cell membrane and recombinant PP2A. Nuclear/cytoplasmic GR ratios under PP2A inhibition by okadaic acid (OA) or overexpression were determined by western-blot.
Results: FM enhanced PP2A activity in both U937 and A549 cells and the effects were not blocked by a β_2-adrenoceptor inhibitor (ICI-118551). FM directly activated PP2A-immunoprecipitates in the membrane and recombinant PP2A. PP2A was detected in GR-immunoprecipitates. PP2A inactivation by OA reduced GR nuclear translocation by BUD and abrogated FM-mediated increase of GR translocation while PP2A overexpression enhanced BUD-induced GR translocation and further increased enhancement of GR translocation by FM.
Conclusions: FM directly activates PP2A in a β_2-adrenoceptor-independent manner. PP2A associated with GR enhances GR nuclear translocation by BUD. This mechanism may contribute to the clinical benefits of BUD+FM combination therapy.

P1778

Effect of fluticasone and formoterol combination therapy on airway remodeling

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Background: Mucosal inflammation, thickening of the basement membrane and increased mass of airway smooth muscle influence asthma airway remodeling. Different cell types contribute to extracellular matrix deposition. Airway smooth muscle cells (ASMC) not only contract and relax but also proliferate, respond to inflammatory stimuli and produce extracellular matrix. As shown previously, glucocorticoids increase the deposition of extracellular matrix by human ASMC under inflammatory conditions.
Methods: To analyse the effect of the β2-agonist formoterol on glucocorticoid-induced extracellular matrix deposition, primary ASMC cultures were set up from asthmatics and non-asthmatic controls. Confluent cells were stimulated with 5% serum with or without a single drug or a combination for a further 72 hr with [3H] proline (0.5microCi). Total extracellular matrix and collagen deposition were monitored by scintillation counts, described earlier.
Results: Compared to non-stimulated cells 5% serum increased matrix and collagen deposition by +42% which was further increased in the presence of 10-8 M glucocorticoids (dexamethasone: +86%, budesonide: +66%, beclomethasone: +59%, fluticasone: +55%). However, 10-8 M formoterol reduced serum-induced matrix and collagen deposition by 36%. In combination, formoterol abolished the stimulating effect of glucocorticoids on matrix and collagen deposition and reduced matrix deposition. This was dose-dependent.
Conclusions: Our data show that β2-agonists combined with glucocorticoids reduce the excessive matrix deposition induced by glucocorticoids alone. Thus, combination therapy may exhibit benefits for asthmatic patients beyond bronchodilating and anti-inflammatory effects.

P1779

AZD3199: A potent and selective β_2-adrenergic receptor agonist with rapid onset of action

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Background: β_2-agonists are standard treatments for asthma and COPD and are varyingly optimized for a number of key pharmacological properties, such as receptor selectivity, systemic exposure, onset of action and duration of effect. AZD3199 is a novel ultra long acting β_2-agonist (uLABA) with improved properties designed to combine 24 hour duration of effect with low systemic exposure and an onset of action similarly rapid to that of formoterol.
Methods: The affinity, potency and efficacy of AZD3199 were measured at human β-adrenergic receptors. Onset of action was measured as relaxation of constricted guinea pig trachea and human bronchial tissue. Activity at the hERG voltage-dependent potassium channel was determined using electrophysiology. Plasma protein binding was measured in multiple species.
Results: AZD3199 was a potent agonist (6 nM EC_{50}) at the human β_2 receptor with an intrinsic activity of 0.8 relative to formoterol. AZD3199 had a rapid onset of action in both guinea pig (22 min) and human (11 min) lung tissue, very similar to formoterol (G-Pig 23 min, human 13 min) and significantly faster than salmeterol (>100 min in both). Similar β_2-agonist activity was seen across multiple species including guinea pig, rat, dog, mouse and rabbit. AZD3199 was highly selective (>1500 fold affinity) for the human β_2 receptor over human β_1 and β_3- receptors with no agonism at either receptor. No activity was seen at the hERG channel at concentrations up to 26 μM. High plasma protein binding (>90%) was seen across multiple species offering the potential for reduced systemic exposure.
Conclusion: AZD3199 is a potent and selective uLABA with an onset of action similar to that of formoterol.

P1780

Oxidative stress-induced corticosteroid insensitivity is reversed by formoterol via inhibition of PI3K signalling in peripheral blood mononuclear cells from patients with COPD and severe asthma

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Rationale: COPD patients show a poor response to corticosteroids which has been linked to oxidative stress. Here we show that the long-acting β_2-agonist formoterol (FM) reversed corticosteroid insensitivity after oxidative stress via inhibition of phosphoinositide-3-kinase (PI3K) signalling.
Methods: The responsiveness to corticosteroids dexamethasone (DEX), budesonide (BUD) and fluticasone propionate (FP) was determined as IC_{50} on TNFα-induced IL-8 release in U937 monocytic cells or peripheral blood mononuclear cells (PBMCs) from patients with COPD or severe asthma.
Results: PBMCs from patients with severe asthma and COPD are less sensitive to DEX compared to healthy volunteers. Although both FM (10^{-9}M) and salmeterol (SM, 10^{-8}M) reversed DEX insensitivity in PBMCs of severe asthma, only FM shows this effect in COPD. In U937 cells, exposure to H_2O_2 decreased BUD and FP sensitivity and increased Akt phosphorylation as a footprint of PI3K activation. FM restored sensitivity to BUD and FP while the effects of SM were weaker and not statistically significant, and FM but not SM, partially inhibited H_2O_2-induced Akt phosphorylation. H_2O_2 also decreased SM-induced cAMP production in U937 cells but did not affect the response to FM. The reduction of SM effects by H_2O_2 was reversed by pre-treatment with a PI3K inhibitor.
Conclusion: These results suggest that FM restored corticosteroid sensitivity via inhibition of PI3K signalling and that a combination of a corticosteroid with FM may be more effective than that with SM in conditions of high oxidative stress, such as in COPD.
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P1781

Endogenous PGE_2 contributes to antigen-induced contractions of guinea pig trachea

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We have previously shown that PGE_2 via EP_1 receptors maintains basal tone of guinea pig trachea (GPT). Our aim was to assess if antagonism of PGE_2 also affected antigen-induced contractions.
Isometric responses to administration of ovalbumin (OVA) were recorded in GPT from guinea pigs sensitized to OVA and expressed as% of the maximal contraction to histamine 100μM. Before challenge with OVA, the selective EP_1 (ONO-8130; 10nM) antagonist was given to naïve preparations as well as together with different combinations of inhibitors and antagonists of histamine (mepyramine and metiamide), leukotrienes (zileuton) and prostaonids (indomethacin).
As shown previously, inhibition of one or two mediator classes, generally had no significant effects, whereas triple mediator antagonism (antihistamines+zileuton+indomethacin) abolished the response to OVA challenge. However, EP_1 antagonism partly reduced the antigen contraction. Moreover, when the EP_1 antagonist was given together with antihistamines and zileuton, the response to OVA was abolished (Table 1).

Table 1. Contraction (% max) to highest OVA dose

Control	75±5
M & M	72±7
INDO	84±5
ONO	59±4*
ZIL	77±1
INDO + M & M	83±1
ONO + M & M	59±10
ZIL + M & M	65±7
ZIL + M & M + INDO	5±3*#
ZIL + M & M + ONO	6±3*#

*p<0.05 vs control, #p<0.05 vs ZIL + M & M.

Effect of indomethacin together with antihistamines and anti-leukotrienes confirms that prostanoids mediate part of the antigen-induced contraction. The new finding is that EP_1 antagonism mimics the effect of indomethacin. This suggests that PGE_2 is the main prostanoid mediating the antigen-induced contractions of GPT, a preparation that is known to respond similarly to human airways where the effects of PGE_2 still await complete delineation.

P1782

Fluticasone propionate inhaled 3 hours after an early allergic reaction partially inhibits the late phase reaction

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Introduction: Inhalation of 800μg budesonide (AJRCCM 1994;149:1447) or 500μg beclomethasone (JACI 1993;91:1163) upon resolution of the allergen-induced early phase response inhibits the late phase asthmatic response (LAR) by 39% and 70%, respectively. This has never been tested for the highly lipophilic corticosteroid fluticasone propionate (FP).
Methods: This randomized double-blinded, placebo-controlled, 3-way crossover study (NCT00716963) was conducted in 6, mild allergic asthmatic patients with a documented dual bronchoconstrictor responses to inhaled allergen. Patients underwent 3 allergen challenges separated by 14 day washout periods. Patients inhaled placebo, 250μg or 1000μg FP at 3 h after allergen challenge, and the magnitude of the LAR was measured at regular intervals until 7 h post challenge. Sputum induction was performed before, 7 h and 24 h post challenge, and methacholine PC20 measured before and 24 h post challenge.
Results: Five patients completed the study. 250μg and 1000μg FP significantly attenuated the LAR compared to placebo; the mean (SD) maximum% fall in FEV1 during the late response was 25.2% (6.2) with placebo, 15.1% (7.1) with 250μg FP (p=0.01), and 18.3% (8.2) with 1000μg FP (p=0.04). Inhibition of the LAR was 40% and 27% for 250μg and 1000μg FP, respectively. There was no effect of FP on allergen-induced sputum eosinophils, with a trend for reduced AHR which was not statistically significant.
Conclusions: FP given three hours after allergen challenge attenuates the LAR, demonstrating that this lipophilic corticosteroid reduces the LAR when administered after allergen exposure.
This research was conducted with support from AstraZeneca.

P1783

Effect of olodaterol on the relaxation of small airways

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Olodaterol (Ol) is a novel, selective, β_2-agonist which offers bronchoprotection in mild asthmatics with duration of action of at least 24h. Small airways are the major site of obstruction in patients with COPD. Thus novel bronchodilators that act on the small airways may be of benefit for COPD. Therefore, this study compared the effect of Ol and formoterol (Fm) on human and rat small airways.
Precision cut lung slices (PCLS) were obtained from rat lungs and human lung tissue obtained following resection. Videomicroscopy was used to measure small airway relaxation.
Rat small airways were contracted to increasing concentrations of carbachol (EC_{50}= 0.11±0.04μM, n=6). Carbachol contraction was then repeated on the same airways following pre-treatment of PCLS with Ol or Fm (1nM). Both agonists significantly ($p<0.05$) inhibited carbachol-induced contraction (Ol: EC_{50}= 0.19±0.05μM and Fm: 0.34±0.07μM, n=6). Carbachol contracted human small airways with an EC_{50} of 0.08±0.03μM (n=6) which was significantly inhibited ($p<0.05$) following pre-treatment with 1nM Ol (EC_{50}= 0.7±0.3μM, n=6) and 1nM Fm (EC_{50}= 1.0±0.6μM, n=6).
Following pre-contraction of rat small airways with 0.1μM carbachol both β_2-agonists induced relaxation ~45% of maximal contraction (n=6). By contrast, carbachol-induced pre-contraction of human small airways was completely reversed by both agonists with EC_{50} values of 4.3±5.7pM (n=6) and 46.1±37.9pM (n=6) for Ol and Fm respectively ($P<0.005$).
Both Ol and Fm had significant bronchodilatory effects on rat and human small airways. Ol was comparable to Fm and showed significantly increased relaxation following partial pre-contraction of human small airways to carbachol.

P1784

Acute reversal of allergen-induced airway hyperresponsiveness (AHR) by olodaterol is synergistically enhanced by tiotropium bromide

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Bronchodilators, like β2-agonists and anticholinergics, are a mainstay therapy for asthma and COPD. Recently, the ultra-long acting β2-agonist olodaterol has been found effective for over 24h in asthma and COPD patients. Increased cholinergic tone, common to these patients, may decrease β2-agonist responsiveness. Using a guinea pig model of allergic asthma, we investigated the reversal of allergen-induced AHR by olodaterol, alone and in combination with the long acting anticholinergic tiotropium bromide. Airway responsiveness was assessed as histamine PC_{100} (provocative concentration increasing pleural pressure by 100%) at baseline (24 h before challenge) and after the early asthmatic reaction (EAR; 5 h after challenge). Phosphate buffered saline (PBS), 1 mM olodaterol and/or 0.1 mM tiotropium (nebulizer concentrations, 3 min) were inhaled at 5.5 h after challenge, followed by PC_{100} determinations at 6.5 h and at 24 h (after the late asthmatic reaction; LAR). Allergen challenge induced AHR to histamine after the EAR (8.3-fold decrease in PC_{100}), which was unaffected by PBS. By contrast, olodaterol strongly reversed the AHR after the EAR (11.4-fold increase in PC_{100}). Tiotropium, which slightly reversed the AHR by itself (2.4-fold), synergistically enhanced the effect of olodaterol to 21.0-fold. After the LAR, the PBS-treated animals were still hyperresponsive (2.3-fold decreased PC_{100}). Tiotropium did not affect this AHR, whereas olodaterol, with or without tiotropium, was still protective. In conclusion, endogenous acetylcholine importantly reduces the reversibility of allergen-induced AHR by β2-agonists.
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P1785

A novel dual-agonist challenge model in guinea pigs for assessment of individual and combined muscarinics antagonists and b_2 adrenoceptor agonists bronchodilator efficacy

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The guinea pig broncoconstriction model has been shown to be predictive for both muscarinics receptor antagonist and b_2-adrenoceptor agonist efficacy in humans. Here we describe a novel *in vivo* model of bronchoconstriction in guinea pigs, stimulated by two different agonists, histamine and methacholine. This dual-agonist challenge model can be used to assess the potency of both M_3 antagonists and b_2-adrenoceptor agonists individually and in combination. This can be extended to investigate compounds with dual activity: Muscarinic receptor antagonists and β_2-adrenoceptor agonists (MABA).
Following administration of test compounds three separate bronchoconstrictor challenges were given to each animal:
The β_2 potency was assessed after challenging guinea pigs with histamine.
Methacholine challenge in the same animal allowed assessment of combined *in vivo* potency at both β_2 and M_3 receptors.
Finally, the potency of the compounds at M_3 receptor alone was assessed using methacholine challenge in the presence of propranolol.
No significant difference in between single- and dual-challenge model was observed for the both M_3 antagonists and b_2-adrenoceptor agonists tested with regards to *in vivo* potency or duration of action, therefore validating our approach. In addition to reducing number of animals used, this approach allowed us to assess individual and combined potency and duration of action for both M_3 antagonists and b_2-adrenoceptor agonists in single animal. This novel bronchoconstriction model provides a robust mechanism for the future testing of MABA compounds.

P1786

Neutraligands of CXCL12: Anti-inflammatory activity in an allergic model of asthma

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Introduction: The chemokine CXCL12 plays an important role in inflammation. Our team identified a small molecule neutralizing CXCL12, belonging to the family of chalcone, named C05. C05 inhibits interaction with its receptors, CXCR4 and CXCR7 (Hachet-Haas et al, JBC 283,23189,2008), and the eosinophil infiltration in a mouse model of allergic eosinophilic airway inflammation. We here evaluated the effect of this neutraligand C05 on airway hyperresponsiveness (AHR), inflammation and airway remodelling.
Methods: Nine week-old male Balb/c mice were sensitized to ovalbumin (OVA+Alum i.p., D0, D7) and challenged to OVA or solvent i.n. on D18,19,20,21. C05 (350µmol/kg) *vs* vehicle was administered i.p. once daily, 2h before each OVA challenge.
Results: OVA induced AHR (whole body plethysmography), eosinophilia, increase in IL-4, IL-5 and mucus secretion in bronchoalveolar lavage fluid, increase in lung collagen, as well as increased IgE and IgG1 in plasma. C05 decreased AHR (44±2%), eosinophilia (48±7%), IL-5 (44±8%), mucus (67±10%) in BAL, and lung collagen (100±21%). IgE and IgG1 levels in plasma and IL-4 secretion in BAL were not modified. In addition, C05 did not modify body or spleen weight. Furthermore, C05 did not induce any CXCR4+ cell recruitment in blood as opposed to AMD3100, a CXCR4 antagonist *vs* control group (11±1, 30±3 *vs* 9±1%).
Conclusion: The CXCL12 neutraligand therefore appears as a safe and good candidate in this asthma model.

P1787

Anti-inflammatory effects of garenoxacin on IL-8 production and ERK1/2 activation induced by lipopolysaccharides in A549 and THP-1 cells

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Objective: The anti-inflammatory properties of macrolides have been applied to the treatment of inflammatory airway diseases. Although the anti-inflammatory properties of fluoroquinolones have been reported, no reports are available regarding a newly developed fluoroquinolone, garenoxacin (GRNX). To examine the immunomodulatory effect of GRNX, we examined the transcription and secretion of inflammatory cytokines by human airway epithelial cells and monocytes stimulated with lipopolysaccharide (LPS).
Methods: A human lung epithelial cell line (A549) and a human monocyte cell line (THP-1) were stimulated with LPS and exposed to different concentrations of GRNX. The transcription of interleukin 8 (IL-8) at 3 h was measured in cell lysates using real-time PCR. The secretion of IL-8 was measured in the supernatants of the cell cultures at 24 h (A549 cells) or 9 h (THP-1 cells) using an enzyme-linked immunosorbent assay.
Results: LPS stimulation resulted in a significant increase in the transcription and secretion of IL-8 by A549 and THP-1 cells. Treatment with GRNX significantly inhibited the transcription and secretion of IL-8 by LPS-stimulated cells through inhibition of LPS-induced ERK1/2 phosphorylation.
Conclusions: GRNX has an anti-inflammatory activity through its capacity to alter the secretion of IL-8 from A549 and THP-1 cell lines.

P1788

Polymerized type I collagen reverts airway hyperresponsiveness development in a guinea pig asthma model

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Recently, polymerized type I collagen (PolC) has been shown to display anti-inflammatory and anti-fibrotic properties in an asthma model. The effect of PolC in the pathophysiology of asthma is unknown. Our aim was to study the effects of PolC in airway obstruction and responsiveness in a guinea pig model of allergic asthma with remodelled airways. After an initial sensitization protocol, guinea pigs were intermittently exposed to allergen (ovalbumin, OA) applied every 10 days for up to 125 days (asthma model group), receiving a total of 12 OA challenges. The control group received saline solution instead of OA. Some animals from both asthma model and control groups were treated with 0.66 mg/ml PolC aerosols administered every 5 days from day 65 to 120. Airway responsiveness to histamine was evaluated before the first OA challenge and at the sixth and twelfth OA challenges. From the first challenge on, OA induced a transient airway obstruction and a progressive rise in baseline Penh (a broncho-obstruction index), measured by barometric plethysmography, which was not modified by PolC treatments. At the sixth challenge, OA-induced hyperresponsiveness was abolished at twelfth OA challenge by PolC treatment. In a separate guinea pig group euthanized at the sixth OA challenge, airway subepithelial fibrosis (determined by morphometry) and granulocyte infiltration were observed. PolC treatment reduced both, granulocyte and fibrosis observed at the twelfth challenge. Our data suggest that the rise of Penh baseline is not induced by airway fibrosis, and that PolC is a biodrug which might become a pharmacological tool to reduce fibrosis, inflammation and hyperresponsiveness in asthma.

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Reduces inflammatory parameters in airways of diabetic-antigen sensitized guinea pigs

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It is well established that there is a negative association between asthma and type I diabetes and relative lack of insulin in an organism results in an overall reduction in inflammatory reactions. This study was planned to determine the inflammatory events in antigen sensitized diabetic guinea pigs. Twenty-five male guinea pigs were categorized into five groups of five each as follows: diabetic, antigen sensitized, diabetic-antigen sensitized, insulin-treated diabetic-antigen sensitized and control animals. Induction of experimental diabetes and antigen sensitization were performed by injection of streptozotocin and ovalbumine, respectively. Animals were killed by exsanguination and bronchoalveolar lavage (BAL) was performed. BAL fluid cellular and protein contents were determined. Airway responsiveness to acetylcholine was assessed. Histopathological examinations were performed on the lungs. Decreases in the airway reactivity in diabetic-antigen sensitized animals were found compared to antigen sensitized animals. Experimental diabetes decreased antigen-induced protein leakage into the airspace as well as the accumulation of inflammatory cells in BAL fluid of antigen sensitized animals. Histopathological results showed that coinduction of experimental diabetes significantly reduced the number of eosinophils in the lungs of antigen sensitized animals. Treatment with insulin completely reversed all mentioned results in the antigen sensitized diabetic animals. Experimental diabetes causes were found to decrease the airway reactivity and inflammatory responsiveness induced by antigen sensitization due to a reduction in the insulin levels.

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Pharmacological assessment of the effects of SB-705498 on capsaicin-induced responses in healthy volunteers and patients with non-allergic rhinitis

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Rationale: "Nasal hyper-responsiveness" has been proposed as a key mechanism driving nasal symptoms in patients with non-allergic rhinitits (NAR). Here, we explore the potential clinical use of the selective TRPV1 antagonist, SB-705498, for treatment of symptoms of rhinitis.
Methods: Two clinical studies were conducted to assess the effect of SB-705498 on nasal responses to incrementally dosed, unilateral intranasal capsaicin challenge: In the first study, a single 400mg oral dose of SB-705498 was assessed in healthy volunteers (HVT). In the second study, 12mg of SB-705498 was administered topically to patients with non-allergic rhinitis. SB-705498 or placebo was administered according to a randomised, double blind, crossover (HVT) or parallel group (NAR) design. 1 hour post dosing, incremental capsaicin challenge (2.5, 12.5 and 50mg) was performed. Symptom scores, secretion weights, peak nasal inspiratory flow (PNIF) and mediators in nasal secretions were evaluated. Blood samples were collected for pharmacokinetic analysis.
Results: Both studies showed a clear signal for effect of SB-705498 versus placebo on the clinical endpoints following intranasal challenge with capsaicin. The relative dose potency for TSS was 3.33 (1.45, 8.26 95% CI) in HVT and 2.81 (0.78, 10.7 95% CI) in NAR patients. All other clinical endpoints (satisfying parallelism) showed approximately 2-5 fold potency shift, except PNIF in NAR patients.
Conclusion: SB-705498 inhibits capsaicin induced nasal responses in HVT and NAR. SB-705498 has potential for further development as a novel, topical intranasal medicine for treatment of rhinitis.