246. Animal models of asthma and COPD and late-breaking abstracts on RCT in asthma and COPD

P2121

Umeclidinium (GSK573719) dose response and dosing interval in COPD

Alison Church[1], Misba Beerahee[2], Jean Brooks[3], Rashmi Mehta[1], Palvi Shah[3]. *[1]GlaxoSmithKline, Research Triangle Park, Durham, NC, United States; [2]GlaxoSmithKline, GlaxoSmithKline R&D, Stevenage, United Kingdom; [3]GlaxoSmithKline, GlaxoSmithKline, Stockley Park, Uxbridge, Middlesex, United Kingdom*

Introduction: Dose differentiation is important in selecting COPD treatments.
Objective: Characterize umeclidinium (UMEC), a long-acting muscarinic antagonist, dose response in COPD patients.
Methods: Randomized, double blind, placebo controlled, crossover study. Subjects were randomized to a sequence of 3 treatments for 7 days separated by a 10–14 day washout. Four once-daily (OD) UMEC doses (15.6, 31.25, 62.5, 125mcg) or 2 twice-daily (BID) doses (15.6, 31.25mcg) were administered via dry powder inhaler. Tiotropium (18mcg) was an active control. Primary endpoint was morning trough FEV_1 on Day 8; population model analysis was applied with ANCOVA. Serial FEV_1, pharmacokinetics and safety were examined. *Post hoc* analysis of the primary endpoint was performed without one investigative site due to poor study practices.
Results: 163 subjects (mean age 59.5yrs, 52% female) were randomized. Emax dose response in trough FEV_1 was characterized with OD dose ordering of UMEC 125>62.5>31.25=15.6mcg. A high potency ED50 (37mcg, 95% CI: 18–57, OD regimen) was estimated. *Post hoc* results were similar. 125mcg OD had more consistent increases in FEV_1 from baseline across serial timepoints over 24h compared with other UMEC doses and tiotropium. No advantage of BID over OD dosing was observed. Drug absorption and elimination were rapid. AEs were highest with UMEC 125mcg OD (18%), placebo (8%), tiotropium (4%), other UMEC doses (5–12%). Two non-drug related, non-fatal SAEs (acute respiratory failure, 15.6mcg OD; myocardial infarction, 31.25mcg OD) were reported.
Conclusions: Dose response for umeclidinium was in the order 125 > 62.5 > 31.25 = 15.6mcg; a once-daily dosing interval was confirmed.
GlaxoSmithKline funded (AC4115321; NCT01372410).

P2122

Apolipoprotein A1 (ApoA1) abrogate cigarette smoke induced emphysema in mice

Dojin Kim[1], SeongWoo Park[1], Jae Jeong Shim[2]. *[1]Pulmonology, Soonchunhayng University Bucheon Hospital, Bucheon, Gyeonggi, Republic of Korea; [2]Pulmonology, Koreas University Guro Hospital, Seoul, Republic of Korea*

Rationale: Apolipoprotein A-1 (ApoA1) have anti-inflammatory and antioxidant properties as well as cholesterol efflux.
Objectives: To determine if the expression of human ApoA1 within the lung protect against the development of emphysema.
Methods: Transgenic human ApoA1 mice(ApoA1 TG) were exposed to CS for 6 month and compared to control transgenic mice. Other ApoA1 TG mice were treated with intratracheal elastase in order to generate emphysema. Measurement; Lung inflammation, oxidative injury was measured in the lung. Emphysema was determined by measuring the mean linear intercept(Lm). Proinflammatory cytokines in the BALF were measured by ELISA and analysis of apoptosis using the TUNEL assay.
Results: Compared with control TG mice, ApoA1 TG mice had significantly less lung inflammation, oxidative damage and apoptosis as well as decreased levels of proinflammatory cytokines. ApoA1 attenuated the development of emphysema in both the smoke-induced and elastase-generated models.
Conclusions: Overexpression of ApoA1 prevents CS and elastase induced emphysema in mice. Augmentation of ApoA1 in the lung could be effective for the prevention or treatment of emphysema/COPD.

P2123

Efficacy and safety of once-daily (OD) fluticasone furoate (FF) in patients with persistent asthma: A 24-week randomised trial

Jan Lötvall[1], Eugene Bleecker[2], William Busse[3], Paul O'Byrne[4], Ashley Woodcock[5], Eric Bateman[6], Edward Kerwin[7], Sally Stone[8], Richard Forth[9], Loretta Jacques[8]. *[1]Krefting Research Centre, University of Gothenburg, Sweden; [2]Center for Genomics and Personalized Medicine, Wake Forest University Health Sciences, Winston-Salem, NC, United States; [3]Department of Medicine, University of Wisconsin, Madison, WI, United States; [4]Department of Medicine, Michael G DeGroote School of Medicine, Hamilton, ON, Canada; [5]School of Translational Medicine, University of Manchester, United Kingdom; [6]Department of Medicine, University of Cape Town, South Africa; [7]Allergy & Asthma Center, Clinical Research Institute of Southern Oregon, Medford, OR, United States; [8]Respiratory Medicine Development Centre, GlaxoSmithKline, Uxbridge, United States; [9]Quantitative Sciences Division, GlaxoSmithKline, Research Triangle Park, Durham, NC, United States*

Introduction: The inhaled corticosteroid (ICS) FF is under development as a OD monotherapy for asthma and in combination with the OD long-acting $beta_2$ agonist vilanterol for asthma and COPD.
Objectives: To evaluate the efficacy and safety of FF in patients ≥12 years with persistent asthma uncontrolled on a stable low-to-mid dose of ICS (≤500mcg fluticasone propionate [FP] equivalent total daily dose).
Methods: In this double-blind, double-dummy, placebo-controlled study, patients (N=343; ITT) received FF (100mcg OD in the evening via a new dry powder inhaler; n=114), FP (250mcg twice daily [BD] via DISKUS™; n=114) or placebo (n=115) for 24 weeks. Primary endpoint: trough FEV_1 at 24 weeks. Powered secondary endpoint: change from baseline in % rescue-free 24h periods over 24 weeks. Safety assessments included adverse events (AEs), incidence of severe exacerbations and 24h urinary cortisol (UC) excretion.
Results: FF and FP significantly improved trough FEV_1 compared with placebo (diff. 146mL [p=0.009] and 145mL [p=0.011], respectively). Significantly more % rescue-free 24h periods were reported for FF (14.8) and FP (17.9) than placebo (both p<0.001). Incidence of on-treatment AEs: FF 53%, FP 42%, placebo 40%. Incidence of on-treatment severe asthma exacerbations: FF 3%, FP 2%, placebo 7%. Statistically significant UC suppression was seen with FF (ratio=0.76; p=0.030) and FP (0.77; p=0.036), relative to placebo.
Conclusions: FF 100mcg OD significantly improved trough FEV_1 to a similar extent to FP 250mcg BD and reduced rescue use relative to placebo. FF was well tolerated with a similar AE profile and effect on 24h UC to FP.
Funded by GSK (FFA112059; NCT01159912).

P2124

RC kinase: A novel kinase expressed by alveolar macrophages that may play a role in COPD and IPF

Stefen Boehme[1], John Ludka[1], Danielle DiTirro[1], Ana Lucia Coelho[2], Cory Hogaboam[2], Tai Wei Ly[1], Kevin Bacon[1]. *[1]Department of Biology, Axikin Pharmaceuticals, Inc., San Diego, CA, United States; [2]Department of Pathology, University of Michigan, Ann Arbor, MI, United States*

We have characterized a novel serine/threonine protein kinase, called RC kinase, whose expression is upregulated in COPD patients. Examination of RC kinase mRNA tissue distribution showed a limited expression pattern restricted mainly to the lungs and trachea. Immunohistochemical analysis with a monoclonal antibody revealed expression in CD68+ alveolar macrophage and bronchial epithelial cells. Various cell lines upregulated RC kinase expression upon exposure to cigarette smoke extract, or conditions of oxidative or endoplasmic reticulum stress, and this

correlated with the production of IL-8. In acute (4 day) and sub-chronic (14 day) cigarette smoke-induced murine models of COPD, treatment with either intratracheally delivered RC kinase siRNA or orally administered novel and specific small molecule inhibitors caused a significant reduction in BAL neutrophilia, as well as decreased levels of KC and CCL-20. There was also a marked reduction in the amount of pulmonary inflammation. In a murine adoptive transfer model of idiopathic pulmonary fibrosis, both siRNA and small molecule antagonist treatment significantly inhibited hydroxyproline production, inflammation and cellular and biochemical markers of fibrosis. Taken together, these results strongly suggest that inhibition of RC kinase may provide a novel therapeutic approach for the treatment of COPD and IPF.

P2125

Statins worse pulmonary fibrosis through enhancing NLRP3 inflammasome activation

Jin-Fu Xu[1,2], George R. Washko[2], Hui-Ping Li[1], Augustine M.K. Choi[2], Gary M. Hunninghake[2]. *[1]Department of Pulmonary Medicine, Shanghai Pulmonary Hospital, Tongji University School of Medicine, Shanghai, China; [2]Pulmonary and Critical Care Division, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, United States*

The role of statins is controversial. To evaluate the association between statin use and ILD. We used regression analyses to evaluate the association between statin use and interstitial lung abnormalities (ILA) in a large cohort of smokers from COPDGene. Next, we evaluated the effect of statin pretreatment on bleomycin-induced fibrosis in mice and explored the mechanism behind these observations in vitro. In COPDGene, 38% of subjects with ILA were taking statins compared to 27% of subjects without ILA. Statin use was positively associated in ILA (odds ratio [OR] 1.60, 95% confidence interval [CI] 1.03-2.50, P=0.04) after adjustment for covariates including a history of high cholesterol or coronary artery disease. This association was modified by the hydrophilicity of statin and the age of the subject. Next, we demonstrate that statin administration aggravates lung injury and fibrosis in bleomycin-treated mice. Statin pretreatment enhances caspase-1-mediated immune responses in vivo and in vitro; the latter responses were abolished in bone marrow-derived macrophages (BMDM) isolated from Nlrp3-/- and Casp1-/- mice. Finally, we provide further insights by demonstrating that statins enhance NLRP3-inflammasome activation by increasing mitochondrial reactive oxygen species generation in macrophages. Statin use is associated with ILA among smokers in the COPDGene study and enhances bleomycin-induced lung inflammation and fibrosis in the mouse through a mechanism involving enhanced NLRP3-inflammasome activation. Our findings suggest that clinicians should be aware that radiological evidence of ILD can develop in some COPD patients treated with statins.

P2126

Effects of combination of PI3Kγ and δ inhibitors on airway hyperresponsiveness in tobacco smoke-exposed mice

Yasuo Kizawa[1], Genki Kimura[1], Keitaro Ueda[1], Yuji Watanabe[1], Shouichi Eto[1], Tadashi Kusama[1], Kazuhiro Ito[2]. *[1]Physiology and Anatomy, Nihon University School of Pharmacy, Funabashi, Chiba, Japan; [2]Airway Disease Section, NHLI, Imperial College, London, United Kingdom*

PI3K δ and γ are known to be involved in inflammatory cell functions. We recently found upregulation of PI3Kδ in lung tissue of COPD patients and ability of a PI3Kδ inhibitor on restoration of steroid sensitivity in airway inflammation in tobacco-smoke (TS) exposed mice. Superior effects of combination of PI3Kγ and δ inhibitors to each inhibitor alone on airway inflammation in TS-exposed mice were also observed. The aim of this study is to evaluate role of PI3Kγ and PI3Kδ on airway hyperresponsiveness (AHR) in TS-exposed mice. A/J mice were exposed to TS for 11 days and IC87114 (IC), AS604850 (AS) and/or fluticasone propionate (FP) were administered intranasally twice a day for 3 days after the last TS exposure. Airway responsiveness was determined as the increment of airway resistance (Δ[sRaw/TV]) before and 1 min after histamine inhalation at 24 h after the last drug dosing. The effects of the PI3K inhibitors on the contractile response to carbachol in guinea-pig tracheal smooth muscle preparation were also evaluated by the isometric tension recording. The concentration-response curve of carbachol was shifted to rightward and reduced the maximal response by AS (10-100 μM), in contrast, the effects of IC (100 μM) was limited in the tracheal smooth muscle. The AHR induced by TS was significantly reduced by AS (4 mg/ml; by 56% inhibition) and IC (4 mg/ml; 43%) alone. The inhibitory effects were enhanced by combination treatment of AS and IC (69%). Moreover, the combination of IC and FP showed stronger inhibition (96%) on the AHR.Considering with our previous findings, the combination of a PI3Kδ or PI3Kγ/δ inhibitor with corticosteroid may offer potential treatment of COPD.

P2127

A robust translational model of acute exacerbations in the tobacco-smoke and poly IC treated mouse

Vincent Russell, Paul Woodman, Andrew Connolly, Dianne Spicer, Joanna Dlugozima, Alan Young. *Pharmacology, Argenta, Stoke Court, Slough, United Kingdom*

Exposure to tobacco smoke (TS) for 4 days induces steroid-insensitive lung inflammation in mice. The effect of adding the viral mimetic poly IC (PIC) to TS-exposed mice was examined.

Methods: Mice were exposed daily to either TS or air for 4d. Saline or PIC was administered intra-nasally. The time course of lung inflammation was examined 4-120hrs after the last exposure and cell numbers measured in the BAL fluid. The acute effects of oral Dexamethasone (DEX 0.3mg/kg) or Roflumilast (ROF 5mg/kg) on the peak inflammation were examined. The effects of DEX on the kinetics of the enhanced inflammation were also examined.

Results: TS caused a lung inflammation which was inhibited by ROF but not by DEX. PIC alone induced an inflammation that was not inhibited by DEX or ROF. Dosing PIC in addition to TS induced an exaggerated response that was significantly greater than the additive effect of the two stimuli. The enhanced response peaked 24hrs after the last exposure then slowly declined. Neutrophils were predominant over the first 48 hrs. Macrophage numbers increased at 24-72hrs and lymphocyte numbers peaked at 48-72hrs. The peak inflammation after TS/PIC exposure was significantly inhibited by ROF (53%, $p<0.05$) and DEX (56%, $p<0.05$), in contrast to the lack of efficacy of DEX against TS or PIC alone. A single dose of DEX after the last exposure reduced the exaggerated response over the entire 120hr study period, but did not fully resolve the inflammation.

Conclusions: TS exposure for 4 days induced a steroid-insensitive lung inflammation. Addition of PIC markedly enhanced the inflammatory response which was sensitive to both steroids and roflumilast, mimicking features of human COPD.

P2128

Inhaled cationic salts modulate macrophage function to reduce inflammation during LPS induced lung injury

S.P. Arold, E.L. Berry, D. Rosa, F.A. Saia, S. Kong, P.L. Wright, R.W. Clarke, D.L. Hava. *Research, Pulmatrix, Lexington, MA, United States*

Pulmatrix is developing PUR118 as a host-targeted, dry powder therapy based on the inhalation of calcium salts for acute exacerbation (AE) control in chronic obstructive pulmonary disease and other inflammatory lung disease. Preclinical data suggest that this approach is effective against an array of pathogens and also reduces inflammation resulting from environmental stimuli such as tobacco smoke. We hypothesized that this treatment could be efficacious in reducing lipopolysaccharide (LPS) induced lung inflammation by modulating the function of pulmonary macrophages. Mice were exposed to nebulized LPS (Pseudomonas aeruginosa) and PUR118, was delivered via whole body exposure 1h post-LPS challenge. Four hours after LPS exposure inflammatory cell counts and chemokine and cytokine concentrations were determined in BAL. PUR118 treatment decreased total inflammatory cell counts and neutrophil counts in the BAL fluid of LPS challenged mice and correlated with reduced KC, IL-6 and TNF-α in BAL fluid. Separately, peritoneal macrophages were isolated from naïve mice and challenged with LPS in media supplemented with calcium to simulate conditions thought to be found in lung fluid lining after PUR118 treatment. Inflammatory mediator secretion and gene expression were determined 2h post LPS exposure. Macrophages stimulated with LPS in the presence of calcium exhibited a dose dependent decrease in KC, IL-6 and TNF-α secretion as well as reduced gene expression for these inflammatory mediators. These data suggest that PUR118 can act through macrophages to reduce lung inflammation and may reduce the risk of AE caused by infections during chronic lung disease.

P2129

Inhaled calcium salts reduce expression of inflammatory mediators associated with tobacco smoke exposure to reduce airway inflammation

P.L. Wright[1], P. Woodman[2], D. Spicer[2], J. Kenyon[1], P. Okerholm[1], V. Russell[2], R.W. Clarke[1], D.L. Hava[1]. *[1]Research, Pulmatrix, Lexington, MA, United States; [2]Respiratory, Argenta, Slough, United Kingdom*

PUR118, an inhaled calcium based dry powder (DP) formulation exhibits preclinical anti-inflammatory and anti-infective activity. PUR118 may provide a novel approach for acute exacerbation control in patients with COPD and CF where the combination of underlying inflammation and pathogen infection result in reduced lung function and quality of life. The goal of this study was to evaluate the impact of PUR118 on gene expression in lung samples from a tobacco smoke (TS) exposure model. Mice were exposed to TS for 4d and treated with PUR118 or DP control 1h prior to TS. Mice were euthanized 4h after the last TS exposure and BAL and lung RNA were collected for cell counts, protein levels and QPCR analyses. Expression of 336 genes was evaluated using targeted QPCR arrays. TS exposure increased BAL cell counts that were reduced with PUR118 (79% reduction in neutrophils; $p<0.001$) to similar levels as a p38 MAPK inhibitor. TS exposure upregulated 21 genes more than 2-fold compared to control mice not exposed to TS and PUR118 treatment inhibited the expression of 11 of these 21 genes. Ten out of the 11 downregulated genes were validated using independent QPCR with 5 significantly inhibited by PUR118 ($p<0.05$). Among genes found

downregulated with PUR118 treatment, many were associated with neutrophilic inflammation including: KC, MIP2, ENA78, IL-6, and MCP-1. BAL protein levels of several of these were similarly reduced by PUR118 compared to controls. Thus, PUR118 diminishes the inflammatory signals induced by TS exposure including many key drivers of neutrophilic inflammation at both the gene and protein level as a mechanism to reduce airway inflammation.

P2130

Protection against allergen-induced airway hyperresponsiveness (AHR) by olodaterol in guinea pigs is synergistically enhanced by tiotropium

Marieke Smit[1,2], Annet Zuidhof[1,2], Sophie Bos[1,2], Harm Maarsingh[1,2], Reinoud Gosens[1,2], Johan Zaagsma[1,2], Herman Meurs[1,2]. *[1]Department of Molecular Pharmacology, University of Groningen, Netherlands; [2]Groningen Research Institute for Asthma and COPD, University of Groningen, Netherlands*

The ultra-long acting β_2-agonist olodaterol has shown to be effective in asthma and COPD. Increased cholinergic tone, common to these diseases, may reduce β_2-agonist responsiveness. In a guinea pig model of asthma, we investigated the protection of allergen (ovalbumin, OA)-induced AHR by olodaterol, alone and in combination with the long acting anticholinergic tiotropium. Airway responsiveness (PC_{100}) was assessed at baseline (24h before OA) and after the early (EAR, 6h after OA) and late (LAR, 24h after OA) asthmatic reactions. 1h before OA, animals were treated with PBS (control), 1 mM olodaterol and/or 0.1 mM tiotropium (nebulizer concentrations, 3 min). OA induced AHR to histamine after the EAR (4.9-fold decrease in PC_{100} compared to baseline), which was fully protected by olodaterol (2.3-fold *increase* in PC_{100}) and tiotropium (1.3-fold *increase*). When combined, a synergistic 4.8-fold *increase* in PC_{100} was observed. After the LAR, AHR (2.8-fold decrease), was also protected by olodaterol, tiotropium and their combination (1.5-, 1.3- and 1.6-fold *increase* in PC_{100}, respectively). OA-induced infiltration of inflammatory cells, measured by BAL after the LAR, was not affected by any treatment. In conclusion, in a guinea pig model of asthma olodaterol and tiotropium protect against allergen-induced AHR after the EAR and LAR, without affecting inflammatory cell influx. Synergism between the drugs was found after the EAR, indicating that acetylcholine reduces the effectiveness of the β_2-agonist and that the combination of olodaterol and tiotropium may be beneficial in the treatment of allergic asthma. (supported by Boehringer Ingelheim Pharma).

P2131

Effects of N-acetylcysteine on airway inflammation, airway hyperresponsiveness and abnormal lung function in chronic ozone-induced COPD model

Feng Li[1], Cornelis Wiegman[2], Joanna Seiffert[3], Pankaj Bhavsar[4], Fan Chung[5]. *[1]Airway Disease Section, National Heart and Lung Institute, Imperial College, London, United Kingdom; [2]Airway Disease Section, National Heart and Lung Institute, Imperial College, London, United Kingdom; [3]Airway Disease Section, National Heart and Lung Institute, Imperial College, London, United Kingdom; [4]Airway Disease Section, National Heart and Lung Institute, Imperial College, London, United Kingdom; [5]Airway Disease Section, National Heart and Lung Institute, Imperial College, London, United Kingdom*

Background: Chronic exposure to ozone in mice induces chronic lung inflammation and emphysema, features of COPD. We determined to examine the preventive and therapeutic effects of N-acetylcysteine (NAC) on airway inflammation, airway hyperresponsiveness (AHR) and abnormal lung function.
Method: C57/BL6 mice were exposed to air or ozone (3ppm, 3h), twice a week over 6 wk, and then ozone exposure was discontinued for 6 wk. NAC treatment (100mg/kg, i.p, twice a week, for 6 wk) was carried out during exposure period or cessation period. Pulmonary function and airway responsiveness were measured and total cells and neutrophils cell in BALF were counted.
Results: Compared with air exposed mice, lung volume indices TLC, FRC, FVC were increased and expiratory indices FEV25/FVC, FEV50/FVC were decreased in ozone exposed mice and continued after ozone cessation. NAC treatment during exposure period or cessation period did not inhibit or reverse abnormal lung function. AHR was induced in ozone exposed mice and persisted after ozone cessation. NAC inhibited AHR during cessation period. Total cells and neutrophils in BALF were increased in ozone exposed mice and returned to normal after ozone cessation. NAC given during exposure period reduced the total cell counts, but not the neutrophil counts.
Conclusions: AHR and abnormal lung function persisted in ozone induced COPD model despite cessation of ozone exposure. Though NAC had no effect on neutrophilic inflammation or abnormal lung function in ozone-exposed mice, it did inhibit AHR during cessation period. NAC interferes with airway smooth muscle dysfunction caused by chronic oxidative stress.

P2132

Interaction of the glutamatergic and nitrergic signaling system in the airway hyperreactivity

Anna Strapkova, Martina Antosova. *Department of Pharmacology, Comenius University, Jessenius Faculty of Medicine, Martin, Slovakia (Slovak Republic)*

It is only little information regarding a possible interaction of glutamatergic and nitrergic system in the airways hyperractivity (AHR). We investigated the effect of agents modulating the activity of these systems on the experimental ovalbumin-induced AHR as well as on the changes of exhaled nitric oxide (eNO) levels. We used the agonists of NMDA receptors - N-methyl-D-aspartic acid (NMDA) and monosodium glutamate (MSG), selective competitive antagonist (DL-2-amino-5-phosphonovaleric acid – AP-5) and selective non-competitive antagonist (dizocilpine - MK-801) of these receptors. We used also non-specific inhibitor of NO synthases N-omega-nitro-L-arginine metylester (L-NAME). The AHR to histamine or acetylcholine was evaluated in in vitro conditions. NMDA administration caused the increase of tracheal smooth muscle response in ovalbumin-induced HR to acetylcholine. The effect of MSG was less pronounced. MK-801 as well as AP-5 provoked the decrease of reactivity mainly to acetylcholine in tracheal smooth muscle, while the former, non-competitive antagonist was more effective. We recorded the changes in eNO levels. The activation of NMDA receptor with NMDA or MSG increased eNO levels. The inhibition of NO synthase with L-NAME caused the fall of eNO levels. We suppose here the participation of constitutive isoforms of NO synthases mainly. MK-801 shows the more expressive effect on the eNO levels during sensitisation than AP-5 group. The results bring a whole new look regarding the relationship of the glutamatergic and nitrergic system in the airway inflammatory diseases.
Supported by Centre of experimental and clinical respirology II, VEGA 14/0010/10, MZ SR 2007/46-UK-11.

P2133

Effect of muscarinic receptors inhibition on cytokine release and inflammatory cells infiltration in the airway of cat as an animal model for COPD

Saeed Kolahian[1], Amir Ali Shahbazfar[2], Khalil Ansarin[3], Hossein Tayefi[1], Hamid Ghasemi[1], Amir Hossein Rashidi[1], Mohsen Hanifeh[4]. *[1]Department of Basic Sciences, Faculty of Veterinary Medicine, University of Tabriz, Islamic Republic of Iran; [2]Department of Pathobiology, Faculty of Veterinary Medicine, University of Tabriz, Islamic Republic of Iran; [3]Tabriz Tuberculosis and Lung Disease Research Center, Faculty of Medicine, Tabriz Medical University, Tabriz, Islamic Republic of Iran; [4]Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Tabriz, Islamic Republic of Iran*

COPD is currently high ranked as the leading cause of death in the world. In this study, fifteen healthy adult male cats were randomly categorized into three groups of five each: I) control, II) exposure to cigarette (COPD model) and III) exposure to cigarette (COPD model treated with tiotropium). They were exposed to cigarette smoke for four days, using 190 cigarettes for each one totally. In group III, cats were treated with one capsule of tiotropium once a day using endotracheal tubes. On the 5th day, the animals in all groups were killed by exsanguination and the lungs were lavaged. In BAL fluid, cells were counted on a hemocytometer. Differential cell counts were made by light microscopy. Cytokines were measured in BALF using Elisa kits. Exposure to cigarette smoke significantly increased the release of IL-8, IL-6, TNFa and MCP-1 in BAL fluid. This increase was significantly reduced by administration of tiotropium. Exposure to cigarette smoke significantly increased total inflammatory cell number in BALF. This increase was significantly attenuated by administration of tiotropium. The numbers of macrophages, eosinophils, neutrophils and lymphocytes were all increased in exposure to cigarette smoke COPD group. Similar to the effect on total cell number, increase in differential cell numbers was reduced by administration of tiotropium. Experimental cigarette smoke-induced COPD causes were found to increase cytokine release and cells infiltration in the airway of cat as an animal model for COPD. It seems that treatment with antimuscarinic agent like tiotropium may attenuate inflammatory events in the airway of this animal model.

P2134

Anti-inflammatory activity of doxofylline and theophylline in LPS-induced lung inflammation

Yanira Riffo-Vasquez, Clive Page. *Sackler Institute of Pulmonary Pharmacology, King's College London, United Kingdom*

Doxofylline and theophylline are two xanthine drugs that show both bronchodilator and anti-inflammatory actions. Data has suggested that doxofylline has a wider therapeutic window than theophylline however, the precise mechanism of action of doxofylline is unknown and its anti-inflammatory activity has not been widely investigated.
Methods: Doxofylline (0.3 mg/kg i.p.) and Theophylline (10 mg/kg, i.p.) were given -24, -1 and 6 h after LPS (10 μg/mice, i.n.) in Balb/c mice. Lung lavage was performed 24 h later. In other experiments, doxofylline (0.3 mg/kg, i.p.) was given -24, -1 and 6 h after injection of LPS (10 μg) into the scrotal sac. Mice were prepared for intravital microscopy 24h later.
Results: LPS recruited significantly higher number of neutrophils (PMN) to the lung (mean±SEM) compared to saline (saline:0±0 *vs* LPS:2.4±0.2 $x10^6$cells/ml, n =4). Doxofylline (Doxo) significantly inhibited the recruitment of PMN (LPS/Doxo:1.4±0.2 $x10^6$cells/ml, n =4; $p<0.05$ *vs* LPS alone). Theophylline (Theo) did not alter the recruitment of PMN in response to LPS (LPS:1.9±0.2 $x10^6$cells/ml *vs* LPS/Theo:2.5±0.2 $x10^6$cells/ml, n=8). Mice showed an accumulation of cells in the tissue (cells/50μm^2) (saline:0±0 *vs* LPS:9.3±2.5, n=4; $p<0.05$) and higher number of cells rolling (cells x100μm^2) in 30 sec (saline:0.2±0.2 *vs* LPS:5.3±2.4, n =4, $p<0.05$) 24 h after LPS injection into the scrotal sac. Doxofylline significantly inhibited cell migration in response to LPS

(LPS/Doxo:3.1±1.9, n=4; p<0.05 *vs* LPS alone) but significantly increased cell rolling (10.2±3.0, n=4; p<0.05 *vs* LPS alone).
Conclusion: Doxofylline significantly reduced cell transmigration in response to LPS, supporting an anti-inflammatory action.

P2135
Protective effect of fenspiride on bronchi in rats with COPD
Nataliya Kuzubova[1], Elena Lebedeva[1], Anatoliy Fedin[2], Ivetta Dvorakovskaya[1]. *[1]Laboratory of Chronic Obstructive Lung Pathology, Research Institute of Pulmonology at I.P.Pavlov's State Medical University, St. Petersburg, Russian Federation; [2]Laboratory of Comparative Respiratory Physiology, I.M.Sechenov's Institute of Evolutionary Physiology and Biochemistry, St. Petersburg, Russian Federation*

Corticosteroid resistance in COPD is urgent problem so the search for drugs that have bronchodilator and anti-inflammatory effect at all stages of COPD is needed.
Aim: To study effect of non-corticosteroid drug fenspiride (F) on contractile activity of bronchial smooth muscle (SM) in rats with COPD.
Methods: Model of COPD was induced in rats by nitrogen dioxide (NO_2) exposure (15 ppm, 1,5 h/day, 60 days). F (0,15 or15mg/kg) was administered *per os* daily before exposure to NO_2. Control rats received 0,9% NaCl. Isometric contraction of bronchial segments caused by electrical stimulation (st) of preganglionic nerve or SM was recorded by displacement electromechanical transducer.
Results: Treatment with F at the acute stage of COPD (15 days) prevented the bronchial constrictor effect of NO_2. Contractile reactions of bronchi were lower than in control with st both nerve (89±4%; 107±2% for 0,15 and 15 mg/kg, control 118±5%, p<0,05) and SM (89±3%; 88±4%; 103±3% respectively, p<0,05). Dilatation effect of low dose F was mediated by interaction with capsaicin sensitive C-fibers that prevented the initiation of neurogenic inflammation as evidenced by lack of COPD structural changes in lungs. At stage of COPD (60 days) bronchodilator effect of low dose F did not appear; high dose F caused a greater SM relaxation with st muscle itself (70±3%) than with nerve st (91±2%, p<0,05). Effect of high dose F was mediated not only the afferent component but due to its direct relaxing effect on SM.
Conclusion: Mechanism of F action on bronchial SM depends on its dose. Revealed bronchodilator and anti-inflammatory effect of extremely low dose F can be used for prevention of COPD development in persons exposed to aggressive environmental factors.

P2136
Effects of corticosteroid and montelukast treatment in inflammation in guinea pigs with chronic allergic inflammation
Nathalia Gobbato[1], Flavia Souza[1], Carla Prado[2], Milton Martins[1], Iolanda Tiberio[1], Edna Leick[1]. *[1]Medicine, School of Medicine University of São Paulo, Brazil; [2]Biological Sciences, Universidade Federal de São Paulo, Brazil*

The effects of montelukast or dexamethasone in asthma pathophysiology are barely understood. We evaluated the inflammation and the eosinophilic recruitment in distal lung parenchyma and airway walls in guinea pigs (GP) with chronic allergic inflammation. 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). After 72 hrs of 7th inhalation, GP were anesthetised, lung strips were submitted to histopathological evaluation.On distal parenchyma both montelukast and dexamethasone were effective in reducing the number of eosinophils, RANTES and NF-kB positive cells compared to OVA group (p<0.05). Montelukast was more effective in reducing the eotaxin positive cells compared to dexamethasone treatment (p<0.05). There was a more expressive reduction of IGF-I positive cells in D group compared to M animals (p<0.05). On airway walls, both montelukast and dexamethasone were effective in reducing the number of eosinophils, IGF-I and RANTES positive cells compared to OVA group (p<0.05). Dexamethasone was more effective reducing the number of eotaxin and NF-kB positive cells than Montelukast (p<0.05).
Conclusions: In this animal model, both corticosteroid and montelukast treatments contribute to the control of the inflammatory response in distal lung parenchyma and airway walls. Dexamethasone treatment induced a greater reduction of NF-kB expression in airway walls which suggests one of the mechanisms that explains the higher efficacy of this therapeutic approach.
Supported by: FAPESP, CNPq, LIM-20-HC-FMUSP.

P2137
Effect of low level light therapy (LLLT) on an experimental model of LPS-induced lung inflammation
Ana Paula Ligeiro de Oliveira[1], Rodolfo de Paula Vieira[1], Jean Pierre Schatzmann Peron[2], Eliane Gomes[2], Flavia Mafra de Lima[1], Momtchilo Russo[2], Flavio Aimbire[3]. *[1]Biophotonics, Nove de Julho - UNINOVE, São Paulo, SP, Brazil; [2]Immunology, University of São Paulo - USP, São Paulo, SP, Brazil; [3]Science and Technology, Federal University of São Paulo - UNIFESP, SP, Brazil*

Acute lung injury (ALI) induced by lipopolysaccharide (LPS) exposure is characterized by cellular infiltrate, edema and altered airway responsiveness. Traditional treatments for ALI include strategies of mechanical ventilation and a variety of drugs such as, corticosteroids and other disease-modifying agents. However these conventional therapies may cause important side-effects that compromise long term therapies. In this sense, Low Level Light Therapy (LLLT) have already demonstrated primising data in reducing airway inflammation. Thus, in the present study we investigated the ability of LLLT to modulate neutrophil infiltration to the lungs. For that, Balb/c mice were submitted to daily dosis of 10 mg of LPS for 3 consecutive days. LLLT group were submitted to irradiation daily at 2, 4 and 6 hours after LPS. Controled groups received PBS and were or not irradiated. On day 4, 24 hours after LPS exposure animals were sacrificed and LBA cellularity, cytokine secretion and airway reactivity by FlexiVent were analysed. Our results demonstrate a significant decrease in total cells and neutrophils recovered from the bronchoalveolar lavage (BAL) of LPS-treated animals after LLLT. We also detected reduced amounts of IL-6 but not IL-17 after LLLT. Airway reactivity to metacholine (Mch) also reduced. In conclusion, our data reveals a promising role for LLLT as an alternative therapeutic approach for acute lung inflammation.

P2138
Effect of CCR3 inhibitors on allergic airway responses in ascaris-sensitized cynomolgus monkeys
Edward Barrett[1], Karin Rudolph[1], Chris Royer[1], T. Ly[2], S. Boehme[2], Kevin Bacon[2]. *[1]Respiratory Immunology, Lovelace Respiratory Research Institute, Albuquerque, United States; [2]Pharmacology, Axikin Pharmaceuticals, San Diego, United States*

Aim: CCR3 has historically been associated with the functional responses of eosinophils in various models of allergic disease. The goal of this study was to determine whether pre-treatment with CCR3 inhibitors via oral and inhaled routes attenuates asthma responses in ascaris sensitized *Cynomolgus* monkeys.
Methods: Animals received a CCR3 antagonist given orally (AP0), or by inhalation (AR1) once or twice a day for 10 or up to 21 days prior to inhaled ascaris challenge. Changes in airway function (immediate and methacholine [MCh]) and inflammation (BAL & blood cells) were evaluated.
Results: Oral (AP0 5 mg/kg; BID 10 days) or inhaled (AR1 860 μg; BID 7 days) treatment showed a trend towards a reduced immediate ascaris and MCh response but did not reach statistical significance. A longer oral treatment (AP0 3 mg/kg; QD 20 days) resulted in a significant reduction in both immediate bronchoconstriction and AHR. This was not associated with a consistent effect on BAL or blood eosinophils, but reduced the lymphocyte, macrophage and mast cell numbers. AP0 given in combination with inhaled fluticasone (79 μg BID) did not yield in a significant additive or synergistic effect on airway function but did lead to a greater reduction in BAL and blood eosinophils than AP0 or fluticasone alone.
Conclusion: Treatment with a CCR3 inhibitor in the non-human primates, *Ascaris* model of asthma, shows that a number of critical parameters can be affected which are significantly different to alterations in the recruitment of eosinophils. Overall, these observations suggest that CCR3 inhibition may have more globally-beneficial responses in an asthmatic setting than previously appreciated.

P2139
PK/PD profiles of the CXCL8 decoy protein PA401 after intravenous and intratracheal administration in saline and LPS exposed mice
Tiziana Adage, Angelika Falsone, Robert Doornbos, Mike Bartley, Andreas Kungl. *Pharmacology and Protein Engineering, ProtAffin Biotechnologie AG, Graz, Austria*

Neutrophils play a crucial role in acute and chronic lung diseases including ALI, COPD, CF and severe asthma, and their presence in the lung has been correlated to disease severity and progression. Among the mediators of neutrophil recruitment into the lung CXCL8 is considered the major player.
CXCL8 exerts its chemotactic activity by binding to glycosaminoglycan (GAG) co-receptors on inflamed cells, thus creating a solid-phase haptotactic gradient and being properly presented to GPC receptors CXCR1/2 on neutrophils.
We have engineered higher affinity for GAGs into human CXCL8 obtaining a protein-based competitor for the CXCL8/GAG interaction. By further knocking-out the GPCR domain, we have obtained a decoy protein (PA401) with potent anti-inflammatory characteristics.
PA401 has been tested in murine models of lung inflammation induced by lipopolysaccharide (LPS) showing strong dose-dependent neutrophil reduction in bronchoalveolar lavage fluid (BALF) after intravenous (IV) and subcutaneous (SC) administration.
In the present study we have compared PA401 activity after IV and intratracheal (IT) administration in the same model, using saline exposed mice as control. PA401 plasma levels were also measured to assess pharmacokinetic profiles.
PA401 has strongly reduced BALF neutrophils number after IV and IT administration (up to -76%). The blood cells increase due to LPS exposure was also partly normalized by IV, but not IT treatment, possibly due to the differences in plasma exposure.
PA401 is a new biopharmaceutical with a unique mode of action interfering with lung neutrophilic inflammation and with activity after systemic and local delivery to the lung.

P2140

Targeting the IL-1β – IL-17A inflammatory axis for the treatment of viral-induced exacerbations of COPD

Anke Sichelstiel[1,2], Aurélien Trompette[1,2], Koshika Yadava[1,2], Laurent P. Nicod[1,2], Benjamin J. Marsland[1,2]. *[1]Service de Pneumologie, CHUV, Lausanne, Switzerland; [2]Department of Biology and Medicine, UNIL, Lausanne, Switzerland*

Chronic obstructive pulmonary disease (COPD) is one of the world's leading respiratory diseases, projected to be the 3rd leading cause of death by 2030. Acute worsening of the disease can be caused by bacterial and viral infections and is often associated with hospitalization. Although COPD exacerbations have been linked to enhanced recruitment of inflammatory cells, such as neutrophils, and to dysregulation of several inflammatory mediators, treatment predominantly relies on corticosteroid therapy with no therapeutic options available to stop disease progression. IL-1β levels are increased in COPD patients during acute exacerbation (Gessner, C. et al. Respir Med 2005; vol. 99 (10) pp. 1229-40); however, it remains to be determined if this is causative of lung dysfunction and exaggerated inflammation or simply associated with the disease.

We found that the severity of COPD exacerbations, characterized by influx of neutrophils to the bronchoalveolar fluid (BALF) and by measurement of lung function, was reduced in mice lacking IL-1β. At early time points after infection this protective effect was mediated by decreased production of IL-17A by Th17 and γδ T cells. However, at the peak of viral infection, neutrophilic inflammation was independent of IL-17A but dependent on IL-1β signaling. Indeed, neutrophil recruitment at late time points during infection could be abrogated by treatment with the IL-1R antagonist Anakinra (Kineret). These data highlight IL-17A and IL-1β as targets for therapeutic intervention during viral-induced exacerbations of COPD.