

TUESDAY, SEPTEMBER 5TH 2006

349. Recent advances in the treatment of COPD and acute lung injury

P3833

No effect of roflumilast on cardiac repolarization in healthy subjects
R. Hermann¹, G. Lahu¹, A. Huenemeyer¹, D. Knoerzer¹, D. Bethke¹, W. Haverkamp². ¹Altana Pharma AG, Konstanz, Germany; ²Kardiologie, Virchow-Klinikum, Charité, Berlin, Germany

Rationale Roflumilast, an oral, once-daily, selective inhibitor of phosphodiesterase (PDE) 4, is under investigation for the treatment of COPD and asthma. It is well known that non-selective PDE inhibitors exert cardiac side effects. Thus, this study assessed potential effects of roflumilast on cardiac repolarization.

Methods The study had a single-center, randomized, placebo-controlled, parallel-group design. On Day 1, 80 healthy subjects (54 males, 26 females) received either oral moxifloxacin 400 mg (as positive control for QT/QTc prolongation to establish assay sensitivity) or placebo. After a 1-day washout, subjects received either placebo or ascending oral doses of roflumilast 500 µg, 750 µg, and 1000 µg once daily for 14, 7, and 14 days each. QT intervals were measured from serial digital 12-lead ECGs and corrected for heart rate with a Fridericia algorithm (QTc_F). The primary endpoint was the largest mean time-matched change of QTc_F from baseline (Day -1). Safety and tolerability were monitored.

Results Moxifloxacin led to a maximum time-matched change in QTc_F versus baseline of 6.79 ms (90% CI 4.15, 9.43; p<0.05), indicating adequate sensitivity of the ECG method. Repeated administration of roflumilast 500 µg and 1000 µg resulted in maximum QTc_F changes of -3.23 ms (90% CI -6.77, 0.31; n.s.) and -4.81 ms (90% CI -8.61, -1.00; p<0.05) from baseline, confirming absence of any QT/QTc prolongation. There were no changes in other ECG variables or time-intervals, vital signs, or laboratory parameters.

Conclusion Repeated administration of roflumilast in oral doses of up to 1000 µg per day had no effect on cardiac repolarization and overall cardiac safety evaluations in healthy subjects.

P3834

Experimental therapy with zinc-ions diminishes oxidant lung injury in rats
Tatjana N. Preobrazhenskaya, Elena S. Lebedeva. *Laboratory of Experimental Pulmonology, Scientific Research Institute of Pulmonology at Pavlov's State Medical University, St. Petersburg, Russia*

Aim: nitrogen dioxide lung's injury prevention in rats treated with zinc sulphate was studied.

Methods: Male Wistar rats were given 0.1% ZnSO₄ solution (per os) 7 days before and during the period of exposure to NO₂ (20 ppm; 1.5 hr/day; 7 days) - 1 group. The calculated ZnSO₄ dose was 80 mg/kg/day. Control rats (2 group)

were NO₂ exposed but received fresh water. The content of Zn in lung, liver, heart tissues, blood, and bronchoalveolar lavage fluid was examined by atomic absorption spectroscopy. Content of malonic dialdehyde (MDA), ascorbic acid, glutathione (G)-SH, and activity of catalase, G-SH-reductase and G-SH-peroxidase were measured in lung tissue.

Results: Zn-content decreased in all examined tissues by 35-40%, and increased twofold in bronchoalveolar lavage fluid in control rats. In ZnSO₄-treated rats tissue and lavage fluid Zn-contents were at starting point level. The increase of lung MDA content was by 52% (1 group) and 92% (2 group). The activity of G-SH-peroxidase increased by 60% in both groups, G-SH-reductase and catalase activity increased in 1 group. Reduction of ascorbic acid and G-SH content was 20-28% in 1st group and 40-45% in 2nd group. The amelioration of pulmonary microcirculation (blood cells aggregation) and alveoli septa structure were less worsened in ZnSO₄-treated rats.

Conclusion: Membrane protective properties of zinc ions provide partial defence of lung tissue against the nitrogen dioxide oxidative action.

P3835

Effect of neutrophil elastase inhibitor (ONO-5046) and gabexate mesilate on acid-induced lung injury in rats

Sumiko Yoshikawa, Kenji Tsushima, Tomonobu Koizumi, Keishi Kubo. *First Department of Internal Medicine, Shinshu University School of Medicine, Matsumoto, Japan*

Background: A specific neutrophil elastase inhibitor (ONO-5046) has been reported to reduce neutrophil infiltration and cytokine production on acid-aspirated lung injury in rats. A synthetic protease inhibitor (gabexate mesilate) used as an anticoagulant has been found to attenuate activated neutrophil-induced pulmonary vascular injury on endotoxin-induced lung injury in rats.

Purpose: The aim is to examine the synergistic effects of gabexate mesilate and ONO-5046 on acid-aspirated lung injury in rats.

Methods: Animals were ventilated mechanically at a tidal volume of 10 ml/kg, a respiratory rate of 50 breaths/min, oxygen concentration of 0.2 l/min. Normal saline (NS, 2 ml/kg) or hydrochloric acid (HCl, 0.1N 2 ml/kg) was instilled into trachea, and gabexate mesilate (10 mg/kg) or NS was administered intraperitoneally. Thirty minutes before instillation, ONO-5046 (10mg/kg/h) or NS (1 ml/kg/h) was infused continuously into the right jugular vein. Blood samples, bronchoalveolar lavage fluid (BALF) and lung tissue samples were obtained 5 hours after instillation. TNF-α, CINC-1, neutrophil elastase and FDP in peripheral blood, total nuclear cell count, absorbance, albumin, TNF-α, CINC-1 and neutrophil elastase in BALF, and wet-to-dry (W/D) ratio were measured.

Results: HCl aspiration increased neutrophil infiltration, cytokine production and W/D ratio, which were attenuated with ONO-5046. In rats treated with ONO-5046 and gabexate mesilate, these reduced more than in rats with ONO-5046 alone.

Conclusions: These results suggested that administration of ONO-5046 and gabexate mesilate was more effective than that of ONO-5046 alone on acid-aspirated lung injury in rats.

P3836

Lipoxin A₄ regulates bronchial epithelium responses to acid injury

Caroline Bonnans¹, Koichi Fukunaga¹, Marilyn A. Levy², Bruce D. Levy¹. ¹Pulmonary and Critical Care Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, United States; ²Cell Biology and Physiology, Washington University School of Medicine, St Louis, MI, United States

Aspiration of gastric acid commonly injures airway epithelium and, if severe, can lead to acute lung injury (ALI). Here, we present evidence for a protective role for prostaglandin E₂ (PGE₂) and lipoxin A₄ (LXA₄) in acid-injured airway epithelium repair and inflammatory responses.

Methods: Normal human bronchial epithelial (NHBE) cells were differentiated in an air/liquid interface system for 21 days, and transiently exposed to acid (0.1N HCl, pH 1.5). After acid removal and pH correction with fresh media, the cells were cultured for up to 72h to determine their response to injury. Morphological changes in NHBE were determined by transmission electron microscopy (TEM). Cyclooxygenase (COX) expression and activity were measured. Cellular responses including proliferation, cytokine (i.e., IL-6) release and PMN transmigration were determined. Stable transfection of recombinant human (rh) LXA₄ receptor (ALX) cDNA in A549 cells was performed.

Results: TEM revealed selective injury to superficial epithelial cells with disruption of cell attachments and cell shedding that was substantially resolved within 6h. Acid increased COX-2-derived PGE₂ production by 2h, and acid-induced PGE₂ significantly increased epithelial ALX expression. LXA₄ increased basal epithelial cell proliferation, potentially blocked acid-triggered IL-6 release and PMN transmigration across NHBE. Expression of rhALX in A549 cells confirmed ALX-dependent inhibition of cytokine release by LXA₄.

Conclusion: Together, these findings indicate that injured bronchial epithelial cells upregulate ALX in a COX-2 dependent manner to promote LXA₄-mediated resolution of airway injury and inflammation.

TUESDAY, SEPTEMBER 5TH 2006

P3837**Low systemic exposure of NVA237, an inhaled once-daily anticholinergic bronchodilator, in healthy humans**R. Thomas¹, E. Eltringham¹, R. Tansley², D. Snape². ¹Simbec Research Ltd, Merthyr Tydfil, United Kingdom; ²Arakis Ltd, Cambridge, Cambridgeshire, United Kingdom

Rationale: A randomized, open-label, crossover study was conducted to assess the pharmacokinetics of inhaled NVA237 and intravenous (IV) NVA237 in healthy humans.

Methods: 16 males received a single IV bolus injection of NVA237 250µg and a single dose of NVA237 250µg via single-dose dry-powder inhaler, separated by a washout of ≥4 days. Blood samples were taken predose and at 2, 5, 10, 15 and 30 min and 1, 1.5, 2, 4, 5, 6 and 8h post-dose.

Results: Mean C_{max} for IV NVA237 was 23.82ng/mL vs 0.62ng/mL for inhaled NVA237. Both IV and inhaled NVA237 reached C_{max} by the first sampling time (2 min). Median AUC₀₋₄ and AUC_{0-∞} for IV NVA237 were 5.28 & 5.42ng/mL.h, respectively, and for inhaled NVA237 were 0.45 & 0.58ng/mL.h, respectively. Median t_[frac12] was 1.5h for IV NVA237 and 2.6h for inhaled NVA237. Relative to the IV dose, the bioavailability of inhaled NVA237 was approx 10%.

Fewer AEs were reported with inhaled than with IV NVA237. Most AEs were mild, with no serious or severe AEs. Dry mouth was reported on 6 occasions with IV NVA237 but did not occur with inhaled NVA237. No clinically significant changes were seen in vital signs and lab tests.

Conclusion: Inhaled NVA237 demonstrated low systemic exposure compared to the IV dose (10% bioavailability). In this study, inhaled NVA237 was not associated with typical systemic anticholinergic AEs such as dry mouth, suggesting a favourable safety profile for this once-daily anticholinergic bronchodilator.

Funded by Arakis Ltd and Vectura Group Plc.

P3838**Pharmacokinetics (PK) and pharmacodynamics (PD) of tetomilast in stable COPD subjects**Suresh Mallikaarjun, Susan Shoaf, Peter Zhang, Kosuke Mitsui, Nestor A. Molfino. *Clinical Development, Otsuka Maryland Research Institute, Rockville, MD, United States*

To evaluate the PK/PD of tetomilast, we conducted a double blind, placebo-controlled, single-dose, cross-over study in 16 COPD subjects. Period 1 comprised Day -2 through Day 7, and Period 2 comprised Day 8 through Day 17. On Day 1 of Period 1, subjects were randomized to receive a single dose of either 50 mg of tetomilast orally with 3.5 mL of placebo solution given as an intravenous (IV) push, or 3.5 mL of solution containing 35 mg of tetomilast given as an IV push with placebo tablets PO. On Day 10 of Period 2, subjects crossed-over. Blood samples were collected for tetomilast concentrations and serial pulmonary function tests were performed over 12 hours. The most commonly reported TEAEs after oral administration of tetomilast 50 mg were headache (20.0%), nausea (13.3%), vomiting (13.3%), and hypertension (13.3%). At 1.5 hours postdose following a single oral 50-mg dose of tetomilast, the mean values of FEV₁ and FVC were statistically significantly (p < 0.05) increased compared to baseline. The bioavailability of oral tetomilast is approximately 100%. Key PK and PD parameters are listed below:

| Parameter | Tetomilast 50 mg PO | Tetomilast 35 mg IV |
|----------------------------|---------------------|---------------------|
| Cmax (ng/mL) | 5.69 ± 1.20 | 7.00 ± 1.93 |
| tmax (h) | 2.75 (1.00-4.37) | 0.13 (0.07-0.75) |
| t1/2,z (h) | 22.9 ± 13.7 | 20.9 ± 9.4 |
| AUC _∞ (ng.h/mL) | 140 ± 74.0 | 91.6 ± 39.2 |
| FEV1 AUC 0-12 (L) | 0.06 ± 0.94 | -0.08 ± 0.92 |
| Maximal FEV1b (L) | 0.08 ± 0.11 | 0.02 ± 0.12 |
| Maximal FVCb (L) | 0.12 ± 0.15 | 0.04 ± 0.17 |

a: Values are median (range); b: Maximum of the (treatment-baseline) point-to-point differences

P3839**Tiotropium reduces subjectively reported sputum production in COPD**J. Powrie, M. Wilkinson, C. Donaldson, A. Wedzicha. *Academic Respiratory Medicine, Royal Free Hospital, London, United Kingdom*

Chronic sputum production in COPD is associated with reduced quality of life, increased exacerbation frequency and faster decline in lung function. It is not known whether modifying sputum production alters disease progression or exacerbation frequency. Tiotropium has been shown to reduce exacerbation frequency but its effect on sputum production is unknown.

142 patients (FEV₁ 1.3l, FVC 2.2l, 55 pack years, 58.4% current smokers, 75% on inhaled corticosteroids) took part in a one year placebo controlled study to assess the effect of tiotropium on sputum inflammatory markers and exacerbation frequency. Tiotropium or placebo was added to usual therapy. 123 patients who were on study for at least 6 months were asked to subjectively assess changes in sputum production. They reported whether sputum production had decreased, increased or remained unchanged over the course of the study.

Tiotropium was associated with subjectively reduced sputum production in 33% of patients compared to 7.9% on placebo (chi², p=0.001). This effect was greater

| | Tiotropium | Placebo |
|------------------|------------|------------|
| number | 60 | 63 |
| less sputum | 20 (33.3%) | 5 (7.9%) |
| unchanged sputum | 35 (58.3%) | 45 (71.4%) |
| more sputum | 5 (8.4%) | 13 (20.6%) |

in ex-smokers (53.8% v 13.3%, p<0.01) than current smokers (16.1% v 3.1%, p=0.08).

Tiotropium reduces subjectively reported sputum production in COPD in both smokers and ex-smokers. Sputum production is difficult to accurately quantify but this subjective reduction in sputum production may represent decreased mucus secretion and a possible mechanism whereby tiotropium might reduce exacerbation frequency and improve quality of life.

P3840**Effects of low dose inhaled carbon monoxide in patients with COPD**D. Bathoorn¹, J. Slebos¹, S. Postma¹, A.J.M. van Oosterhout², M. Kerstjens¹.

¹Groningen Research Institute for Asthma And COPD, Department of Pulmonology, University Medical Center, Groningen, The Netherlands; ²Laboratorium of Allergology & Pulmonary Diseases, University Medical Center, University of Groningen, Groningen, The Netherlands

Introduction: *In vitro* and *in vivo* studies have shown that carbon monoxide (CO) has both anti-inflammatory and anti-oxidant capacities. CO could therefore be of therapeutic use in COPD, a disease which is pathophysiologically characterised by inflammation and oxidative stress.

Aim: To investigate the effects and feasibility of low dose CO inhalation in stable COPD patients.

Methods: On 4 consecutive days, 3 stable COPD patients (GOLD I-III, ex-smokers, males, median age 76, packyears 31, no asthma) inhaled 95ppm CO for two hours. We measured lung function, PC₂₀ methacholine, and inflammation in induced sputum at baseline, and at the morning after the 4 inhalation sessions. HbCO-levels were measured every half hour during inhalation.

Results: Effects on FEV₁, PC₂₀ and HbCO are shown in the table. The median changes in sputum inflammatory cells (post CO inhalation minus baseline values) were: total cells -3.9 *10⁶/ml, neutrophil% -9.8, eosinophil% -0.2, macrophage% 10.5, lymphocyte% 1.0. No adverse events occurred

Pilot results

| Patient | FEV ₁ * (L) | FEV ₁ post CO (L) | PC ₂₀ * (L) | PC ₂₀ post CO | HbCO%* max. | HbCO% post CO |
|---------|------------------------|------------------------------|------------------------|--------------------------|-------------|---------------|
| #1 | 3.1 | 3.2 | 26 | 39.6 | 0.3 | 3.2 |
| #2 | 1.7 | 1.7 | 0.55 | 3.65 | 0.2 | 3.0 |
| #3 | 1.4 | 1.7 | 0.038 | n/a | 0.1 | 2.7 |

*baseline value

Conclusion: In this pilot, the directions of the effects of CO inhalation on both lung function and sputum inflammation are encouraging. Inhalation of CO led to maximal HbCO levels of 3.2%. Patients sustained the pilot very well. These promising results have led to a formal RCT.

Funded by Stichting Astma Bestrijding.

P3841**Is the hypoxic effect of formoterol really linked to a shunt-like effect?**S. Centanni¹, F. Di Marco¹, P. Santus¹, M. Verga¹, A. Pistone¹, M. Cazzola².

¹Unit of Pneumology, Univ of Milan, S Paolo Hosp, Milan, Italy; ²Unit of Pneumology, Cardarelli Hosp, Naples, Italy

The administration of β₂-agonists to patients with airways obstruction often results in a transient decrease in PaO₂ despite concomitant bronchodilation. This has been attributed to the pulmonary vasodilator action of these agents, increasing blood flow to poorly ventilated lung regions and thus increasing ventilation-perfusion inequality, a shunt-like effect. We have investigated the acute effects of formoterol on the arterial blood gas tensions of 30 patients with COPD. Sixty min after the inhalation of formoterol 12 µg, P(A-a)O₂ rose significantly (4.50 mmHg) and, as expected, showed a highly significant negative correlation (r=-0.91; p<0.001) with the fall in PaO₂ (-4.79 mmHg). This finding was not linked to hypoventilation because PaCO₂ was not elevated. The expected raise in FEV₁ (0.19 L) elicited by formoterol showed a significant negative correlation with the fall in PaO₂ (r=-0.36; p=0.04), whereas the changes in IC (0.37 L) and TGV (-0.73 L) did not correlate with the changes in PaO₂(IC: r= 0.017, p=0.93; TGV: r= -0.014, p=0.94). Also DL_{CO}, VA, and DL/VA rose from baseline (1.48 mL/min¹/mmHg, 0.25 L, and 0.11 mL/min¹/mmHg, respectively), although their raises were not correlated with the fall in PaO₂ (DL_{CO}: r=0.09, p=0.63; VA: r=0.10, p=0.59; DL/VA: r=0.11, p=0.58). These findings suggest a primary true shunt effect in inducing the hypoxic effect of formoterol, although the contribution of an impairment of the ventilation-perfusion cannot be excluded.

P3842**Effect of flavonoids extracted from *Cleome Arabica* on human neutrophil elastase activity**

Rachid Kacem. *Biological Sciences, Faculty of Sciences, Ferhat Abbas University, Setif, Algeria*

Introduction: *Cleome Arabica* was used in herbal medicine for the treatment of COPD. Extracts from this medicinal plant as reported by many scientists were found to be enriched by many bioactive molecules including flavonoids. There was no research work carried out to investigate the effect of bioactive molecules which present in the extract of this medicinal plant on Human Neutrophil Elastase (HNE) activity.

Aim: This study was aimed mainly to investigate the effect of flavonoids extracted from *Cleome Arabica* on HNE activity.

Methods: flavonoids extracted from *Cleome Arabica* by a method developed in our laboratory based on gradual extraction using organic solvents. Total flavonoids concentration was estimated. Analysis of separated flavonoids was realized by HPLC and TLC. Inhibition of HNE activity was tested using Elastin Nutrient Agar test and microplate assay.

Results: Results of this study clearly indicating that extracts from *Cleome Arabica* inhibited HNE activity. SWex4 was found to be the most active fraction. This fraction was analyzed by HPLC and identification of flavonoids was realized using TLC technique on silica gel. The results revealed the detection of 2 Flavonols; Rutin (Flavonol glycoside) and Quercetin. These compounds could be the active biomolecules responsible for the marked inhibition of HNE activity.

Conclusion: TLC analysis of active fraction (SWex4) revealed the detection of 2 Flavonols Rutin (flavonol glycoside) and Quercetin responsible for the marked inhibition of HNE activity.

P3843**Relation of low serum lipid levels with coronary atherosclerosis in COPD patients**

Hasan Ergen¹, Sema Sarac¹, Atilla Saygili¹, Zeliha Arslan². ¹*Chest Diseases, Sureyyepasa Chest and Cardiovascular Diseases Training and Research Hospital, Istanbul, Turkey;* ²*Chest Diseases, Adiyaman State Hospital, Adiyaman, Turkey*

COPD is one of the most common reasons of mortality and morbidity in the world. It is well known that the incidence of coronary atherosclerosis in COPD patients is low. Our aim in this study was to search for low lipid levels in COPD patients and to evaluate it as a reason for lower incidence rates of coronary atherosclerosis. 100 male patients hospitalized at our hospital between July 2004 and August 2005 were enrolled in the study. 81 of these patients were severe COPD patients with FEV1 <50%, 19 were moderate COPD patients with FEV1 50-69%. 50 healthy volunteers from the same age group served as the control group. Zon type spirometry was used for pulmonary function evaluation and Behring dimension autoanalyser for biochemical measurements. SPSS for Windows 10.0 program was used for statistical analysis. Quantitative data with normal distribution were evaluated using student's t test and data without normal distribution were evaluated using Mann-Whitney U test.

When patients and control groups were compared with respect to smoking history, triglyceride, cholesterol, LDL-cholesterol, LDL/HDL levels there was a statistical significance ($p < 0.001$), but the difference was not significant with respect to age and cholesterol levels ($p > 0.05$).

We concluded that serum lipid levels were significantly lower in COPD patients and that might be one of the reasons for low incidence of coronary artery disease in COPD patients.

P3844**Modulation of sputum gene expression in COPD by fluticasone/salmeterol**

E. Rand Sutherland, Taylor A. Moss, Allen D. Stevens, Juno Pak, Richard J. Martin. *Medicine, National Jewish Medical & Research Center, Denver, CO, United States*

Introduction: The extent to which fluticasone and salmeterol modify airway inflammation in mild-to-moderate chronic obstructive pulmonary disease (COPD) is not well-defined. We compared the ability of salmeterol and fluticasone to modify gene expression in induced sputum of subjects with mild-to-moderate COPD.

Methods: Subjects with mild-to-moderate COPD were randomly allocated to 6 weeks of either salmeterol, 50mcg bid (S) or fluticasone, 250mcg/salmeterol, 50mcg bid (F/S). Physiology and sputum induction were obtained before and after treatment. Sputum cell counts were performed, RNA was extracted, and real-time reverse-transcription polymerase chain reaction for TNF- α , TGF- β , MMP-12 and CD68 was performed. Cycle thresholds (CT) were normalized for GAPDH and compared pre- and post-treatment.

Results: Fifteen adults with age 61.8 ± 1.8 yr (mean \pm standard error), post-bronchodilator FEV1 $67.4 \pm 3.1\%$ predicted, FEV1/FVC ratio $59.6 \pm 3.0\%$ and 41.2 ± 4.3 pack-years smoking were enrolled. With both treatments, an reduction in neutrophils was observed (S $-7.9 \pm 9.0\%$ and F/S $-5.4 \pm 8.4\%$ ($p = 0.8$), whereas macrophages increased $4.6 \pm 5.4\%$ and $7.3 \pm 5.0\%$ respectively ($p = 0.7$). A significant reduction (CT increase) in expression of TNF- α and TGF- β was observed when F/S was compared with S alone, mean difference of $+1.4 \pm 0.6$ ($p = 0.04$) CT for TNF- α and $+0.73 \pm 0.3$ CT ($p = 0.04$) for TGF- β . MMP-12 expression differed also

($+1.1 \pm 0.6$ CT) but did not meet statistical significance ($p = 0.07$). No significant differences were observed in CD68 expression.

Conclusion: The addition of an inhaled corticosteroid to a long-acting beta-agonist reduces expression of TNF- α and TGF- β in induced sputum, independent of changes in sputum cellularity.

P3845**Inhibition of human lung fibroblast functions by roflumilast N-oxide**

Silvia Boero, Michela Silvestri, Federica Sabatini, Antonio Nachira, Giovanni A. Rossi. *Pulmonary Disease Unit, G Gaslini Inst, Genoa, Italy*

Lung fibroblasts that contribute to airway remodelling express phosphodiesterase 4 (PDE4). The PDE4 inhibitor roflumilast is currently under investigation for COPD and asthma therapy. We investigated the effect of roflumilast N-oxide, the active metabolite of roflumilast, on ICAM-1 expression, eotaxin release, and α -smooth muscle actin (α SMA) expression in human lung foetal fibroblasts. Lung fibroblasts (cell line GM06114) were pre-incubated with 0.3 nM- 1 μ M roflumilast N-oxide in the presence of 1 nM PGE₂ (for ICAM-1) or 10 - 1000 pg/mL IL-1 β (for α SMA). Cells were then stimulated for 24 h with 5 ng/mL TNF α for ICAM-1 expression and eotaxin release or for 48 h with 1 ng/mL TGF β ₁ for α SMA expression. ICAM-1 expression was assessed by FACS, eotaxin release was assessed by ELISA, and α SMA expression was detected by western blotting. Stimulation with TNF α up-regulated ICAM-1 expression ($p < 0.001$) and increased eotaxin release ($p < 0.05$). Expression of α SMA, a surrogate of myofibroblast differentiation, was stimulated with TGF β ₁. Pre-incubation with roflumilast N-oxide in the presence of PGE₂ concentration-dependently inhibited ICAM-1 expression (IC₅₀ ~ 0.8 nM, $p = 0.001$) by a maximum of about 50% . In parallel, roflumilast N-oxide (≥ 1 nM) decreased eotaxin release ($p < 0.05$). In the presence of IL-1 β (10 pg/mL), roflumilast N-oxide (1 μ M) substantially reduced TGF β ₁-induced α SMA expression by about 50 - 60% . Roflumilast N-oxide, the active metabolite of roflumilast, effectively inhibits *in vitro* lung fibroblast functions involved in the recruitment/activation of inflammatory cells and airway remodeling.

P3846**Inhibition of histone acetyltransferases attenuates cytokine and LPS-induced up-regulation of arginase in rat alveolar macrophages**

Mareille Warnken, Martin Heideking, Sonja Matthiesen, Kurt Racké. *Department of Pharmacology, University of Bonn, Bonn, Germany*

Arginase is markedly up-regulated in inflammatory airway diseases. In alveolar macrophages (AM) arginase is up-regulated by lipopolysaccharides (LPS), which in addition exert permissive effects on other stimuli such as transforming growth factor (TGF- β). Histone acetyltransferases (HATs) play a major role in the regulation of chromatin assembling and gene expression. By testing the effect of the HAT inhibitor anacardic acid (AA) the present study aims to explore the role of HATs in the up-regulation of arginase by LPS and other stimuli.

Rat AM were cultured for 20 h in the absence or presence of LPS, TGF- β , granulocyte-macrophage colony-stimulating factor (GM-CSF) and AA. Thereafter, arginase activity was determined.

Arginase activity in rat AM cultured in the absence of test substances averaged out at 71 ± 5 mU/mg protein ($n = 6$). Presence of LPS (10 ng/ml, a submaximal effective concentration) caused only a moderate increase in arginase activity by 30 ± 8 mU/mg protein. Combination with TGF- β (1 ng/ml) caused a further significant increase by 58 ± 5 mU/mg protein, whereas TGF- β alone showed an enhancement by only 25 ± 5 mU/mg protein. The TGF- β mediated increase in arginase activity in presence of LPS was suppressed by AA (10 - 30 μ M) in a concentration dependent manner to 18 ± 6 mU/mg protein at 30 μ M. Moreover, exposure to GM-CSF (1 - 100 ng/ml) induced a concentration dependent increase in arginase activity up to 252 ± 30 mU/mg protein. Presence of AA (30 μ M) decreased the GM-CSF mediated stimulation of arginase activity to 126 ± 19 mU/mg. In conclusion histone acetylation is involved in cytokine- and LPS-stimulated up-regulation of arginase activity in macrophages.

P3847**Effects of TGF-beta and budesonide on MAPK phosphorylation, IL-6/IL-11 secretion, and cell proliferation in human lung fibroblasts**

Girolamo Pelaia¹, Luca Gallelli¹, Bruno D'Agostino², Alessandro Vatrella³, Giovanni Cuda¹, Donatella Fratto¹, Teresa Renda¹, Umberto Galderisi², Elena Piegari², Nunzio Crimi⁴, Francesco Rossi², Mario Caputi³, Francesco S. Costanzo¹, Carlo Vancheri⁴, Rosario Maselli¹, Serafino A. Marsico³. ¹*Department of Experimental and Clinical Medicine, University "Magna Graecia" of Catanzaro, Catanzaro, Italy;* ²*Department of Experimental Medicine, Section of Pharmacology, Second University of Naples, Naples, Italy;* ³*Department of Cardiothoracic and Respiratory Sciences, Second University of Naples, Naples, Italy;* ⁴*Department of Internal and Specialistic Medicine, University of Catania, Catania, Italy*

Transforming growth factor-beta (TGF-beta) is crucially involved in the fibrotic events characterizing interstitial lung diseases, as well as in the airway remodeling process typical of asthma. Within such a context, the aim of our study was to investigate, in primary cultures of normal human lung fibroblasts, the effects of TGF-beta1 on mitogen activated protein kinase (MAPK) phosphorylation, cell

TUESDAY, SEPTEMBER 5TH 2006

proliferation and production of interleukins 6 (IL-6) and 11 (IL-11), in the presence or absence of a pretreatment with either budesonide or MAPK inhibitors.

MAPK phosphorylation was detected by Western blotting, using specific monoclonal antibodies against the phosphorylated, active forms of these enzymes. Cell count was performed by Trypan blue staining, and the release of IL-6 and IL-11 into cell culture supernatants was assessed by ELISA.

TGF-beta1 (10 ng/mL) significantly stimulated MAPK phosphorylation ($p < 0.01$), and also enhanced cell numbers as well as the secretion of both IL-6 and IL-11 ($p < 0.01$), which reached the highest increases at the 72nd hour of cell exposure to this growth factor. All such effects were prevented by budesonide (10^{-8} M) and, with the exception of IL-6 release, also by a mixture of MAPK inhibitors.

Therefore, our findings suggest that the fibrotic action exerted by TGF-beta1 in both airways and lung parenchyma is mediated at least in part by MAPK activation and by an increased synthesis of the profibrogenic cytokines IL-6 and IL-11; these effects seem to be prevented by glucocorticoids via inhibition of MAPK phosphorylation.

P3848**Expression of transforming growth factors beta (1, 2, 3) and connective tissue growth factor in the peripheral lung from GOLD stage 1-2 COPD patients and smokers with normal lung function**

Gaetano Caramori¹, Margaret McLeish², Andrew Lewis², Phillip Monk², Paolo Casolari¹, Kazuhiro Ito³, Kian F. Chung³, Ian M. Adcock³, Peter J. Barnes³, Alberto Papi¹. ¹Centro di Ricerca su Asma e BPCO, University of Ferrara, Ferrara, Italy; ²Cambridge Antibody Technology, Cambridge Antibody Technology, Cambridge, United Kingdom; ³Airway Disease Section, NHLI, Imperial College London, London, United Kingdom

Transforming growth factor (TGF)- β 1 is highly expressed in epithelium and macrophages of small airways in patients with chronic obstructive pulmonary disease (COPD). It is a potent inducer of fibrosis, partly *via* the release of the potent fibrogenic mediator, connective tissue growth factor (CTGF). Two other members (2 and 3) of TGF β family may also be pro-fibrogenic *in vitro*. However, the expression of TGF β 2 and 3, and CTGF in the peripheral lung of patients with mild to moderate chronic obstructive pulmonary disease (COPD) has not been previously studied. We have investigated the expression of TGF β 1, 2, 3, and CTGF in lung parenchyma of smokers with mild to moderate (GOLD stages 1 and 2) COPD (n=16) compared with age-matched smokers with normal lung function (n=18). Peripheral lung tissue was obtained during lung resection surgery. We examined formalin-fixed paraffin-embedded lung sections. By immunohistochemistry (IHC) we detected TGF β 1, 2, 3, and CTGF expression mainly in bronchiolar epithelial cells and macrophages. The semiquantitative IHC scoring for TGF β 1, 2, 3, and CTGF in bronchiolar epithelial cells and endoalveolar macrophages was not significantly different between COPD patients and smokers with normal lung function. These data suggest that TGF β 1, 2, 3, and CTGF expression are not increased in the peripheral lung of GOLD stage 1 and 2 COPD patients. This abstract is funded by Cambridge Antibody Technology (UK).

P3849**MUC5AC and MUC5B expression in central airways from non-smokers, smokers with normal lung function and GOLD stage 1 and 2 COPD patients**

Gaetano Caramori¹, Paolo Casolari¹, Marina Saetta², Simonetta Baraldo², Kazuhiro Ito³, Leonardo M. Fabbri⁴, Peter J. Barnes³, Ian M. Adcock³, Giorgio Cavallero⁵, Kian F. Chung³, Alberto Papi¹. ¹Centro di Ricerca su Asma e BPCO, University of Ferrara, Ferrara, Italy; ²Section of Respiratory Diseases, University of Padova, Padova, Italy; ³Airway Disease Section, NHLI, Imperial College, London, United Kingdom; ⁴Section of Respiratory Diseases, University of Modena, Modena, Italy; ⁵Thoracic Surgery, University of Ferrara, Ferrara, Italy

Mucus expectoration is a common feature of chronic obstructive pulmonary disease (COPD). Sputum is mainly produced in central airways. The two major mucins in sputum are MUC5AC and MUC5B. The major site of mucus production in central airways is represented by the submucosal glands. The expression of MUC5AC and MUC5B in bronchial epithelium and submucosal glands from patients with COPD is not known. We investigated by immunohistochemistry (IHC) the expression of MUC5B and MUC5AC in bronchial rings (obtained at lung resection surgery) from 10 asymptomatic non-smoking subjects, twenty smokers with normal lung function and twenty smokers with COPD. Quantitative analysis of the immunohistochemical staining of bronchial epithelial cells and submucosal glands was performed using dedicated computerised digital software. The total area covered by MUC5AC +ve cells in the bronchial epithelium was increased in smokers (with and without COPD) ($66.1 \pm 5.7\%$ total epithelial area) compared to non-smoking subjects ($21.2 \pm 7.1\%$ $p < 0.01$). The total area covered by MUC5AC+ve cells in the bronchial submucosal glands was significantly higher in patients with COPD ($22.2 \pm 5.5\%$ total gland area) compared to smokers with normal lung function ($10.6 \pm 2.1\%$; $p < 0.05$) and non-smoking subjects ($5.9 \pm 3.5\%$; $p < 0.05$). MUC5B expression was not significantly different between groups both in bronchial epithelium and submucosal glands. These results suggest that COPD is associated with increased expression of MUC5AC in the bronchial airways, particularly in bronchial glands. This may be involved in the pathogenesis of the disease. Funded by ARCA, FerraraRicerche and MIUR ex 60%.

P3850**Roflumilast but not methylprednisolone inhibited cigarette smoke-induced pulmonary inflammation in guinea pigs**

F. Fitzgerald¹, D. Spicer¹, A.E. McAulay¹, L. Wollin², R. Beume².

¹Pharmacology Department, Argenta Discovery, Stoke Poges, United Kingdom;

²Altana Pharma AG, Konstanz, Germany

Rationale In COPD, the inflammatory processes that result from cigarette smoking are largely resistant to corticosteroid therapy. Roflumilast, an oral phosphodiesterase 4 (PDE4) inhibitor with anti-inflammatory properties, is under investigation for the treatment of COPD. This study assessed the effects of roflumilast and the corticosteroid methylprednisolone in a guinea pig COPD model of cigarette smoke (CS)-induced pulmonary inflammation.

Methods On Days 1 to 11, animals were exposed to CS (5 cigarettes/day) and received either placebo, oral roflumilast 0.4 mg/kg once daily, or oral methylprednisolone 10 mg/kg twice daily. Cell counts and protein concentrations were determined in bronchoalveolar lavage (BAL) fluid 1 h after last CS exposure.

Results In placebo-treated animals, 11 days of CS exposure led to a 1.5-fold increase in inflammatory cells and a 1.8-fold increase in protein concentrations in BAL fluid. Roflumilast inhibited the CS-induced accumulation of total cells by 97%, neutrophils by 75%, eosinophils by 120%, lymphocytes by 66% (each $p < 0.01$), and macrophages by 68% (n.s.) vs placebo. Protein concentration was reduced by 70% ($p < 0.01$). Methylprednisolone showed only minor inhibitory activity on protein concentration and CS-induced cell influx, but inhibited accumulation of eosinophils to levels below baseline ($p < 0.01$) as compared to placebo.

Conclusions In this animal model of COPD, the PDE4 inhibitor roflumilast reduced pulmonary inflammatory cells that are relevant to COPD, whereas the corticosteroid methylprednisolone only decreased the number of eosinophils. This study demonstrates the potential of roflumilast as broad anti-inflammatory therapy for COPD.