395. Lung injury and repair: reactive oxygen species and beyond

P3767
The role of pre-receptor glucocorticoid metabolism in regulating the severity of ALI
Sian Lax[1], Micheal Wilson[2], Masao Takata[2], David Thickett[1]. *[1]Respiratory Medicine, University of Birmingham, United Kingdom; [2]Surgery and Cancer, Imperial College, London, United Kingdom*

Acute lung injury (ALI) is a major cause of respiratory failure in the critically ill patient. With a mortality rate of 40-60%, 50% of survivors left with pulmonary impairment and no current licenced treatment there is a need for novel therapies. Our current research suggests that local steroid metabolism by alveolar macrophages is defective in ALI patients. As a major function of these cells is phagocytosis of apoptotic neutrophils during resolution of inflammation, we sought to investigate the effect of pre-receptor glucocorticoid metabolism in a murine model of ALI.
Using intra-tracheal instillations of LPS, we analysed the inflammatory response in wildtype mice compared to those deficient in 11β-hydroxysteroid dehydrogenase 1 (HSD1). These mice specifically lack the enzyme which converts inactive cortisone to active cortisol. Cell infiltrates and expression of several inflammatory markers within bronchial lavage fluid, as well as tissue permeability were examined to evaluate the immune response and lung damage.
Here we report that in HSD1$^{-/-}$ mice, lung permeability increases ($6.1x10^{-3}$ $\pm 0.9x10^{-3}$ v $10.7x10^{-3}$ $\pm 2x10^{-3}$) 24hrs post-LPS. In addition, there was a build-up of apoptotic neutrophils ($20.3x10^{3}$ $\pm 5.9x10^{3}$ v $39.2x10^{3}$ $\pm 6.1x10^{3}$), with a significant increase in $CD11c^{+}CD11b^{+}$ monocytes recruited into the lung ($35.2x10^{3}$ $\pm 5.7x10^{3}$ v $81.8x10^{3}$ $\pm 1.4x10^{3}$) 72hrs post-LPS. Moreover, dys-regulation of IL6 was observed (1.5ng/ml ±0.62 versus 0.2ng/ml ±0.08) 72hrs post-LPS.
Our data indicate that insufficient alveolar glucocorticoid metabolism augments lung injury and suggests that therapies targetting defective macrophage HSD-1 expression may have value in ALI.

P3768
Amelioration of hyperoxia-induced lung injury in newborn mice using a sphingolipid-based intervention
Jeroen Tibboel[1,3], Stephen Joza[1,2], Irwin Reiss[3], Johan C. de Jongste[3], Martin Post[1,2]. *[1]Dept. of Physiology and Experimental Medicine, Hospital for Sick Children, Toronto, ON, Canada; [2]Dept. of Laboratory Medicine and Pathobiology, University of Toronto, ON, Canada; [3]Dept. of Pediatrics, Erasmus University Medical Center - Sophia Children's Hospital, Rotterdam, Zuid-Holland, Netherlands*

Aim: To characterize lung function and BAL sphingolipid profile of newborn mice during hyperoxia exposure and recovery in room air, and to examine the effect of D-sphingosine supplementation during recovery.
Methods: Newborn mice were exposed to 80% O2 for 4 weeks and allowed to recover in room air for another 4 weeks. Lung function measurements, histological and morphometrical analysis of lung tissue was performed and BAL fluid was collected during hyperoxia and after recovery with and without D-sphingosine supplementation. Sphingolipids in BAL were quantified by tandem mass spectrometry.
Results: Hyperoxia increased lung resistance and decreased compliance, total lung capacity and forced expiratory flow. After recovery, resistance, compliance and total lung capacity had normalized whereas forced flows remained low. Sphingolipids, including ceramides, were significantly increased after hyperoxia. Ceramides were still increased after 2 weeks of recovery, but normalized to control values after 4 weeks. Addition of D-sphingosine during the first 5 days of recovery reduced ceramide levels at 2 weeks and partially reversed the hyperoxia-induced increase in alveolar size and arrest in alveolarization at 4 weeks, although no further improvement in lung function parameters was observed.
Conclusion: Exposure of newborn mice to hyperoxia caused restrictive and obstructive lung function changes that partially recovered in room air, while alveolar morphology remained abnormal. Hyperoxia increased ceramide levels, with normalization after recovery. D-sphingosine addition during recovery reduced ceramide levels and ameliorated hyperoxia-induced alveolar arrest.

P3769
PI3K p110γ is overexpressed in IPF lung tissue and fibroblast cells. In vitro effects of its inhibition
Enrico Conte[1], Elisa Gili[1], Mary Fruciano[1], Martina Korfei[2], Evelina Fagone[1], Maria Iemmolo[1], Nunzio Crimi[1], Andreas Güenther[2], Carlo Vancheri[1]. *[1]Department of Clinical and Molecular Biomedicine, University of Catania, Italy; [2]Department of Internal Medicine, University of Giessen Lung Center, Justus-Liebig-University, Giessen, Germany*

Molecular pathogenesis of Idiopathic Pulmonary Fibrosis (IPF) remains unclear. We recently demonstrated a key role for the PI3K pathway in both proliferation and differentiation into myofibroblasts of lung fibroblasts treated with TGF-β. In this research we assessed the expression of Class I PI3K p110 isoforms in IPF lung tissue and tissue derived fibroblast cell lines. Moreover, we investigated the in vitro effects of the selective inhibition of p110 isoforms on IPF fibroblast proliferation and fibrogenic activity.
To evaluate expression levels of PI3K p110 isoforms, IHC as well as Western blot and Flow Cytometry analysis were performed on normal and IPF lung tissue/fibroblasts. The in vitro effects of selective pharmacological inhibition as well as specific gene silencing by siRNAs were studied in fibroblast cell lines established from both normal and IPF tissues.
No significant differences between normal and IPF tissue/cells were observed for the expression of PI3K p110 α, β and δ isoforms whereas p110γ resulted overexpressed in both IPF lung homogenates and ex-vivo fibroblast cell lines. The IHC results show a strong immunoreactivity for p110γ in myofibroblasts of IPF lungs. Moreover, in pathologic bronchiolar structures of IPF lungs, basal cells exhibited a pronounced nuclear expression of p110γ which was hardly detectable in normal lung tissues. Furthermore, as a consequence of both p110γ pharmacological inhibition and gene silencing, a significant inhibition of proliferation rate and α-SMA expression were observed in IPF fibroblasts whereas no effects were found on normal cells.
Our data indicates that PI3K p110 γ isoform can be a novel pharmacological target.

P3770

Inhibition of the sonic hedgehog pathway at the primary cilium prevents the effect of TGF-beta 1 on alveolar epithelial cells

Elika Farrokhi Moshai[1], Arnaud Mailleux[1], Valérie Besnard[1], Stéphanie Brayer[1], Monique Dehoux[1,2], Bruno Crestani[1,3]. *[1]Inserm U700, UFR de Médecine Xavier Bichat, Paris, France; [2]Laboratoire de Biochimie, Hôpital Bichat, Paris, France; [3]Service de Pneumologie, Hôpital Bichat, Paris, France*

Introduction: The mesenchymal differentiation of alveolar epithelial cells induced by Transforming Growth Factor-beta1 (TGF-beta1), also called Epithelial Mesenchymal Transition (EMT), may contribute to Idiopathic Pulmonary Fibrosis (IPF). The Sonic Hedgehog (SHH) pathway is involved in epithelial cells-fibroblasts interaction during fetal lung development and lung fibrogenesis in adult lung. Previously, our laboratory has demonstrated that the SHH pathway is necessary to the action of TGF-beta 1 in human pulmonary fibroblasts (Cigna et al. in revision).
Aims: We hypothesized that the SHH pathway could play a role in mesenchymal differentiation of alveolar epithelial cells induced by TGF-beta 1.
Methods: The A549 cell line or primary alveolar epithelial murine cells are pre-treated 1h with agonists (recombinant SHH, Smoothened Agonist) or with inhibitors of the pathway (Cyclopamine, HPI-4, GANT61) in the absence or presence of TGF-beta 1 (1-5 ng/ml) for 48h in serum-free medium. The expression of E-Cadherin, N-Cadherin, and fibronectin is evaluated by real-time PCR, Western blotting and immunocytochemistry. The migratory capacity of A549 is also measured in these conditions.
Results: Inhibition of the pathway via SMO/GLI abolishes the effect of TGF-beta 1 on the migration of epithelial cells but do not influence the effect of TGF-beta 1 on cell differentiation. By contrast, the inhibition of the HH pathway in the primary cilium with HPI-4 prevents and reverts the effect of TGF-beta 1 on epithelial cell differentiation.
Conclusions: Our results indicate that the primary cilium controls the effect of TGF-beta 1 on A549 cells in vitro.

P3771

Protective effect of mesenchymal stem cells (MSC) on hypoxia-induced epithelial-mesenchymal transition of alveolar epithelial cells (AEC)

Yurdagul Uzunhan[1], Nadia Randrianarison[1], Dominique Marchant[1], Nicolas Dard[1], Jerôme Larghero[2], Carole Planès[1]. *[1]EA 2363, UFR SMBH, Université Paris 13, PRES Sorbonne Paris Cité, Bobigny, France, Metropolitan; [2]Centre de Thérapie Cellulaire, Hôpital Saint Louis, Paris, France, Metropolitan*

Background: Epithelial-to-mesenchymal transition (EMT) of AEC induced by TGF-β1 or hypoxia (HX) may contribute to pulmonary fibrosis. In animal models of lung fibrosis, administration of allogenic MSC reduced fibrosis and mortality by an unknown mechanism. We hypothesized that MSC may favor alveolar wound healing by preventing hypoxia-induced EMT. We tested in vitro the paracrine effects of MSC on the phenotypic changes of AEC induced by HX.
Methods: Rat AEC cultured on Transwell filters were exposed to HX (3 or 1.5% O2) for up to 12 days in the presence/absence of human MSC on the bottom of the well. Transepithelial electrical resistance (TER) as well as epithelial markers (E-cadherin, ZO-1, TTF-1) and mesenchymal markers (smooth muscle α-actine, vimentin) were assessed by ohm-meter, immunofluorescence and Western blot at d6 and d12.
Results: Exposure of AEC to HX resulted in a dose- and time-dependent decrease of TER and of epithelial marker expression, together with an increase in mesenchymal marker expression. These effects were reproduced by treatment with cobalt chloride and were partially prevented by anti-oxydant drugs (Euk134 or N-acetyl-cystein). Co-culture with MSC (or MSC-conditioned medium) partially prevented the effects of HX regarding TER and phenotypic changes.
Conclusion: These results indicate that MSC alleviate the phenotypic changes of AEC induced by HX in vitro through a paracrine mechanism. Preservation of AEC phenotype by MSC may limit EMT and could partly explain the anti-fibrotic effect of MSC in vivo.
Funded by: College des Enseignants de Pneumologie, Chancellerie des Universites de Paris (Fondation du Legs Poix).

P3772

Hyperoxia mediates barrier permeability dysfunction in 16HBE140- cells

Hanady Al-Shmgani, John Moody, Robert Sneyd, Roy Moate. *School of Biomedical and Biological Sciences, Plymouth University, Plymouth, Devon, United Kingdom School of Biomedical and Biological Sciences, Plymouth University, Plymouth, Devon, United Kingdom Human and Physical Resources, Peninsula College of Medicine and Dentistry, Plymouth, Devon, United Kingdom Electronic Microscopy Centre, Plymouth University, Plymouth, United Kingdom*

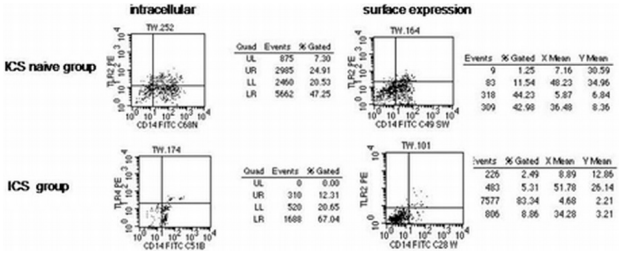
Background: Reactive oxygen species (ROS) induced by hyperoxia have been postulated to be responsible as mediators of oxidative stress and damage in human and experimental animal lung epithelium. This study aimed to determine the effects of hyperoxia on the tight junction associated protein ZO-1 in the human bronchial epithelial cell 16HBE140-, and the possibility of a protective role of antioxidant vitamins C and E.
Methods: 16HBE140- cells were cultured at a density of 2.5×10^5 cell/cm^2 in an air-liquid interface for 6 days where the transepithelial electrical resistance (TER) developed to 230±28 Ω cm^2. The experimental groups were divided into six sets, exposed for 24h to normoxia (21% O_2, 5% CO_2) as a control; hyperoxia (95% O_2, 5% CO_2); hyperoxia with 10^{-7} M vitamin E (α-tocopherol); hyperoxia with 10^{-7} M vitamin C; hyperoxia with 10^{-6} M vitamin C and hyperoxia with a combinations of vitamins E and C (10^{-7}, 10^{-6} M respectively). TER measurement, immunofluorescence staining of ZO-1 and RT-PCR to detect IL8, IL6, TNF-α and ZO-1 expression were used.
Results: The reductions of the TER in hyperoxic groups (hyperoxia and hyperoxia with antioxidants) were associated with the reduction in the ZO-1 thickness and downregulation of ZO-1 expression compared with the control. In contrast, the expression of IL-8, IL-6 and TNF-α was upregulated in the hyperoxic groups compared with the control.
Conclusion: Hyperoxia induced barrier integrity disruption represented by the decrease in TER and the reduction of ZO-1 levels is associated with an increased in the expression of pro-inflammatory cytokines IL-8, IL-6 and TNF-α. The antioxidant vitamins E and C had only a slight protective effect against hyperoxia damage.

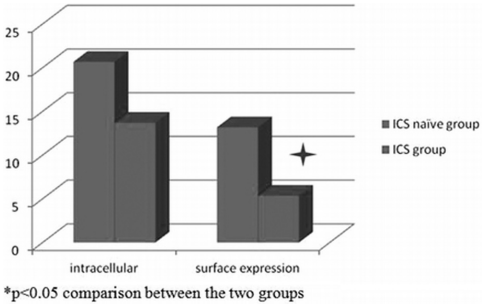
P3773

Long-term use of ICS influence TLR2 expression on induced sputum from severe COPD patients

Haixing Zhu, Guochao Shi, Wei Tang. *Respiratory, Ruijin Hospital, Shanghai, China*

Objective: To evaluate the influence of long-term use of inhaled corticosteroid on TLR2 expression on and in macrophages from induced sputum in severe COPD patients.
Methods: Severe stable smoking male COPD patients treated in OPD of Respirology were divided into2 groups: long-term ICS group (ICS≥500/day futicasone propionate, ≥1 year) (n=21) and ICS naive group(n=29). With flow cytometry, we tested TLR2 expression on macrophages from induced sputum in these COPD patients. We also examined the mRNA expression in the induced sputum.
Result: The TLR2 extracellular expression on the macrophages from induced sputum in long-term ICS treatment group were lower than the ICS naïve group(13.69±1.17 vs 20.12±4.37,p=0.019). The TLR2 intracellular expression (5.35±1.67 vs 12.81±4.89), the TLR2mRNAand TNFαmRNA expression also showed decreased trends in ICS long-term treatment group.





*p<0.05 comparison between the two groups

There were positive relationship between the expression of TLR2 extracellular and intracellular expression.
Conclusion: Long-term use of ICS may have negative influence on TLR2 expression in the airway of COPD patients, which may increase the pneumonia susceptibility in severe COPD patients.

P3774

Early signs of oxidative stress in COPD

Helen Evsyukova[1], Marina Palay[1], Anna Vjushina[2]. *[1]Hospital Therapy, St.Petersburg State University, Medical Faculty, St. Petersburg, Russian Federation; [2]Neurophysiology, I.P. Pavlov Institute of Physiology RASc, St. Petersburg, Russian Federation*

Oxidative modification of proteins is the early and most reliable sign of oxidative stress which plays an important role in pathogenesis of COPD. We studied the oxidative modification of proteins in 12 patients with COPD at the exacerbation of the disease, 12 patients at the remission of COPD and 24 healthy subjects. It was shown that COPD patients had an increased amount of carbonyl derivatives

originating at the initiation of oxidation amino acid rests of protein at the exacerbation (0.05±0.01U/mg) and remission stage (0.03±0.00U/mg) than in control subjects (0.01±0.00U/mg) ($p<0.05$).COPD patients had a decreased amount of carbonyl derivatives at the stage of elongation of the process. This may be due to the considerable protein destruction. We have shown low level of reduced thiols at the exacerbation (0.41±0.03U/mg) and remission stage (0.33±0.03U/mg) in comparison with control subjects (0.52±0.01U/mg) ($p<0.001$) which points out to the exhaustion of antioxidant system. The data received correlate with disruption of pulmonary function and oxygen saturation in COPD patients.

P3775

The influence of roflumilast on the IV-type collagen level in patients with III stage COPD

Mykola Ostrovskyy, Oleksandr Varunkiv, Iryna Savelikhina, Mariana Kulynych-Miskiv. *internal Medicine #3, Ivano-Frankivsk National Medical University, Ivano-Frankivsk, Ukraine*

Background: Chronic obstructive pulmonary diseases are diagnosed in 4-6% of men and 1-3% of women above 40 years old. The persistent inflammatory process in bronchi, the development of microcirculation disorders, the increasing of hypoxia processes result in the activation of fibroblasts and their production of IV-type collagen, which is manifested by the formation of peribronchial pneumosclerosis.
Purpose of the study: The evaluation of the IV-type collagen level dynamics in BALF of patients with 3rd stage COPD during the treatment with Roflumilast.
Materials and methods: The contents of the IV-type collagen in BALF was evaluated in 39 patients with III stage COPD in a relapse stage using enzyme-linked immunosorbent assay before and after 3 months of treatment with the inhibitor of phosphodiesterase -4 – Roflumilast, 500 mg per day, inside, together with the basic 3rd stage COPD treatment (GOLD, 2010).
Results of the study: The contents of the IV-type collagen in BALF before the beginning of treatment in patients with 3rd stage COPD was (69.13±3.12) ng/ml, which is in 7,14 times higher than in almost healthy people, whose contents constituted (9,68±0,54) ng/ml. After the second examination in 90 days, the above-mentioned indicator in patients fell down by 2,45 times and was equal (28,26±2,23) ng/ml.
Conclusions: The depression of IV-type collagen levels demonstrate the ability to slow down pneumosclerosis progression in patients with III COPD who are treated with Roflumilast.

P3776

The effect of intravenous immunoglobulin on oxygen-dependent metabolism of blood cells in patients with community acquired pneumonia

Usman Farkhutdinov, Albert Mirchaidarov, Rafagat Farkhutdinov. *Department of Internal Diseases, City Hospital 21, Ufa, Russian Federation Department of Internal Diseases, City Hospital 21, Ufa, Russian Federation Department of Internal Diseases, Bashkortostan State Medical University, Ufa, Russian Federation*

Active oxygen forms (AOF) determine the microbicidal activity of phagocytes. Their low generation may be one of the reasons of latent inflammatory process.
The aim was to study the effect of i.v. immunoglobulin immunovenin (IMV) on the production of AOF in in-vitro experiments and in patients with community acquired pneumonia (CAP).
Methods: The method of chemiluminescence (ChL) registration was used to study the effect of various doses of IMV (0.005 mg/ml, 0.01 mg/ml, 0.05 mg/ml) on the production of AOF in the blood of healthy subjects. In the clinical part of the research AOF generation was studied in 35 patients with CAP. 17 patients with CAP received standard therapy. In 18 patients IMV was added to the treatment.
Results: In in-vitro experiments IMV dose of 0.005 mg/ml increased ChL intensity of blood by 18.4±0.3% ($p<0.05$) the doses of 0.01 mg/ml and 0.05 mg/ml increased ChL intensity by 48.6±2.1% ($p<0.05$) and 64.5±3.4%($p<0.05$) times respectively. In comparision with normal findings ChL intensity of blood in patients with CAP has been found to be reduced by 1.5 times. The use of IMV in patients has increased production of AOF in blood and resulted in positive dynamics of clinical findings. In the case of standard therapy low ChL intensity of blood maintained, the inflammatory process often had a prolonged course.
Conclusion: IMV may be used in the treatment of patients with CAP owing to its influence on AOF. It allows to increase AOF production by phagocytes and decrease the incidence of latent inflammatory processes.

P3777

Converse airway effects of nicotine *in vitro* and *in vivo*

Yuan Xu, Lars-Olaf Cardell. *Division of Ear, Nose and Throat Diseases, CLINTEC, Karolinska Institutet, Stockholm, Sweden*

Cigarette smoke, which contains high concentrations of nicotine and endotoxin (LPS), plays a pivotal role in the development of asthmatic airway inflammation and hyperreactivity (AHR). But the mechanisms behind this are poorly known. The present study examines the effects of nicotine and LPS on murine airways both *in vitro* and *in vivo*.
In the *in-vitro* model, murine tracheal segments were cultured in presence of nicotine (10 μM) and/or LPS (10 μg/ml) for 4 days. Smooth muscle contractibility was assessed with myograph and inflammatory mediator expressions measured with real-time PCR. In the *in-vivo* model, mice were exposed to nicotine (24 mg/kg/day) via osmotic pumps for 28 days followed by intranasal (i.n.) LPS (1 mg/ml) instillation during the last 3 days. Airway resistance was measured using FlexiVent® after i.v. methacholine challenge and inflammatory cells in the bronchoalveolar lavage fluid were counted.
In vitro, nicotine increased contractions to bradykinin (BK) and des-Arg9-BK. Carbachol contractions only increased after combined nicotine and LPS exposure. Moreover, nicotine specifically up-regulated Toll-like receptor 2 and 4 as well as inflammatory mediators COX-2 and MCP-1. *In vivo*, nicotine had no effects alone, but i.n. LPS caused both AHR and acute pulmonary neutrophillic inflammation. 28-days of *in-vivo* nicotine exposure suppressed the LPS-induced AHR both in central and peripheral airways and prevented pulmonary neutrophil infiltration.
It is interesting to note that the local smooth muscle effect of nicotine differs markedly from the *in-vivo* effect which involves a much more complex system of inflammatory cells and mediators. This is important to acknowledge when evaluating the toxic effect of nicotine.

P3778

Alveolar macrophage activity in experimentally induced pneumonia

Usman Farkhutdinov. *Department of Internal Diseases, City Hospital 21, Ufa, Russian Federation*

Alveolar macrophages (AM) play an important role in lung inflammation.
The aim was to study the AM state in experimentally induced pneumonia (PN).
Methods: PN was induced in 120 white rats by transthoracic injection of pneumococcus culture. The animals were sacrificed at 1, 3, 7 day of PN course. The content of malonic dialdehyde (MDA) was determined in the animal lungs. The production of active oxygen forms (AOF) by alveolar macrophages (AM) was determined by nitroblue tetrazolium (NBT) test. In addition phagocytic activity (PhA) of AM has been assessed, morphological investigations were performed.
Results: The development of PN in rats was accompanied by increase of MDA content in the lungs, by increase of NBT-test values and PhA of AM. The NBT values of AM correlated directly to MDA content in the lungs. Due to morphological changes the animals were divided into 2 groups. In both groups the level of MDA in the lungs remained high. In the 1st group of rats on the 3, 7 days NBT values and PhA of AM were found to be increased, infiltration changes were abundant in the lungs. The rats of the 2nd group have demonstrated lowering of NBT values and PhA of AM, destructive changes in the lungs.
Conclusion: AOF produced by AM initiated free radical processes and lung inflammation in experimentally induced PN. Decreased production of AOF and reduction of PhA of AM demonstrated the inflammatory complications.

P3779

Hydrogen gas alters the production of reactive oxygen species in alveolar epithelial cells *in vitro*

Kenichi Kokubo, Takashi Inoue, Kazuko Yamashita, Toshihiro Shinbo, Minoru Hirose, Hirosuke Kobayashi. *Department of Medical Engineering and Technology, Kitasato University School of Allied Health Sciences, Sagamihara, Kanagawa, Japan*

Aim: The pulmonary toxicity of high concentration of oxygen during mechanical ventilation relates to reactive oxygen species (ROS). Hydrogen gas (H_2) has potential as eliminating highly reactive ROS. We therefore expected that H_2 could reduce the adverse effects of the oxygen exposure. The aim of the present study was to determine the protective effects of H_2 against various oxidative stresses on epithelial cells *in vitro*.
Methods: Human alveolar epithelial cells (A549) were incubated with antimycin A which enhances the generation of superoxide anions (O_2^-) in mitochondria, with menadione which exogenously generates O_2^- and H_2O_2 in the cells, or with Cu^+ (converted from Cu^{2+} by ascorbic acid) which exogenously generates hydroxyl radical by the Fenton reaction with added Cu^+ and endogenous H_2O_2. The viability of the cells as well as the levels of O_2^- and highly reactive ROS in the cells was evaluated with or without 2% H_2.
Results: The viability of the cells incubated with menadione or Cu^+ decreased or did not change in the presence of H_2, respectively, while that with antimycin A significantly increased in the presence of H_2 (n=12, $P<0.01$). The production of O_2^- induced by antimycin A significantly decreased with the addition of H_2 (n=11, $P<0.01$), while highly reactive ROS induced by menadione significantly increased in the cells incubated with H_2 (n=5, $P<0.01$).
Conclusions: H_2 protects alveolar epithelial cells against injury induced by antimycin A probably due to the decrease in the production of O_2^- in mitochondria, while H_2 cannot act protective against ROS induced by menadione or the Fenton reaction, meaning that H_2 cannot overcome the effects of exogenously provided ROS.

P3780

Effect of stress on free radical indices in bronchial asthma

Elena Zaprudnova[1], Svetlana Soodaeva[2], Igor Klimanov[2], Timur Li[3], Yulia Petrovskaya[4], Lidiya Nikitina[4], Fedor Petrovskiy[4]. *[1]Department of Biochemistry, Vladimir State University, Vladimir, Russian Federation; [2]Clinical and Experimental Biophysics, Pulmonology Research Institute, Moscow, Russian Federation; [3]Neurological Department, N.I. Pirogov Russian National Research Medical University, Moscow, Russian Federation; [4]Allergy and Clinical Immunology, Khanty-Mansiysk State Medical Academy, Khanty-Mansiysk, Russian Federation*

We investigated the influence of stress on the levels of some free radical indices in exhaled breath condensate (EBC) at people with asthma (BA) compared with healthy people.

The control group (n=57) included healthy, young people aged 20 - 23 years. The second group (n=62) included young people who suffer from BA (remission) aged 20-23 years.

The levels nitrate/nitrite, metabolites NO, and malondialdehyde (MDA) were determined at rest and under stress in both healthy and in people with BA. Stress state of subjects formed on the background of the educational process, as exam stress is one of the first places among the causes of mental stress in students.

The study found that people suffering from BA, there is increased production of NO in the lungs, which is reflected in increased levels of NO metabolites in EBC by 1.3 times compared with control. Patients with BA most sensitive to stress conditions in comparison with healthy young people, resulting in a higher level (1.3 times) in their production of NO metabolites during stress (5,8±0,03 mkM in patients with BA during stress and 4,5±0,05 mkM in healthy under stress, $p < 0.05$). In patients with BA as an increase in basal level of MDA in a 1.6 times ($p < 0.05$) in EBC in comparison with control. Under conditions of stress has been increasing process of lipid peroxidation, as evidenced by increased concentrations of MDA in EBC of 1.5 times in normal and 1.7 times in patients with BA.

Thus, the most active enhancement of free radical processes in the lungs leads to the conclusion that patients with BA most sensitive to the stress associated with the educational process.

P3781

Regulation of VEGF receptors and co-factors by hypoxia and hyperoxia

Thomas Blythe, Shaney Barratt, Caroline Jarrett, Gavin Welsh, Ann Millar. *Lung Research Group, University of Bristol, United Kingdom*

VEGF exerts its biological effect through specific receptors, VEGFR-1 and VEGFR-2 and co-receptors, neuropilin-1 and neuropilin-2, keading to a complex regulatory sytem. The presence of hypoxia in lung disease and its treatment by high flow oxygen have been proposed to contribute to lung injury. We hypothesised that hypoxia and hyperoxia may lead to changes receptor and co-receptor expression and hence regulation of VEGF bioactivity.

A549 (ATCC) as a model for lung epithelial cells and Human pulmonary microvascular endothelial cells (HMVEC-l) were obtained and cultured in for 24 hours in a PROOX chamber in normoxic (N), hyperoxic 90% O2 (H), hypoxic conditions were represented by culture in the presence of Cobalt chloride ($CoCl_2$). The cells were then lysed for protein or RNA extraction. Expression of VEGFR1, 2 and NRP-1, NRP-2 was established at both mRNA and protein level by q-PCR, immunofluorescence and Western blotting.

No significant changes were detected in A549 expression of VEGF-R1, NRP-2, however NRP1 was significantly reduced compared to control values ($p<0.05$) in the presence of hypoxia and there was non-significant trend to a reduction with both hypoxia and hyperoxia in VEGFR2. In HMVEC-l there was a significant reduction ($p< 0.05$) in the levels of VEGFR2 and NRP-1 and a trend towards reduction in VEGFR1 and NRP-2 with hypoxia.

These results suggest that changes in oxygen tension may have a significant effect on VEGF bioactivity with potential implications for the use of oxygen therapy.

P3782

Peroxiredoxin 6 attenuates lipopolysaccharide-induced plasminogen activatro inhibitor 1 expression by regulating autophagy

Dong Yang, Yuanlin Song, Jiayuan Sun, Tong Lin, Jing Bi, Chunxue Bai. *Respiratory Department, Zhongshan Hospital, Fudan University, Shanghai, China Respiratory Department, Zhongshan Hospital, Fudan University, Shanghai, China Respiratory Department, Zhongshan Hospital, Fudan University, Shanghai, China Respiratory Department, Zhongshan Hospital, Fudan University, Shanghai, China Respiratory Department, Zhongshan Hospital, Fudan University, Shanghai, China Respiratory Department, Zhongshan Hospital, Fudan University, Shanghai, China*

Objective: To evaluate the roles of Peroxiredoxin(Prdx)6 in the expression of plasminogen activator inhibitor(PAI)-1 in lipopolysaccharide(LPS) induced acute lung injury(ALI).

Methods and results: ALI was induced in Prdx6(-/-) and C57BL/6J mice 4hrs or 24hrs after intratracheal instillation of LPS(5mg/kg), characterized by inflammation in morphology, higher wet/dry ratio, elevated protein concentration and increased neutrophils in bronchial alveolar lavage fluid(BALF), which were more significantly in Prdx6(-/-) mice. After LPS administration, PAI-1 mRNAexpressions were markedly increased in a time-dependant manner and the PAI-1 concentration in BALF were markedly increased at 4hrs and decreased nearly to baseline at 24hrs in Prdx6(-/-) mice compared to C57BL/6J mice. Autophagy was significantly enhenced with higher expression of LC3B in Prdx6(-/-) mice compared to C57BL/6J mice. Primary cultured macrophages were stimulated by LPS (10ug/ml) for 4hrs. The level of reactive oxygen species(ROS) in macrophages from Prdx6 (-/-) mice was significantly higher than that from C57BL/6J mice. The release of PAI-1 was significantly increased in macrophages from Prdx6(-/-) mice compared to wildtype mice after LPS instillation. PAI-1 release was partially suppressed by extracellular signal–regulated kinase(ERK) and p38 mitogen-activated protein kinase inhibitor(MAPK) but not by c-Jun N-terminal kinase inhibitors.

Conclusions: In LPS-induced ALI, Prdx6(-/-) mice increased PAI-1 expressions of partially dependent on enhanced autophagy in lungs and p38 MAPK and ERK in macrophages. Thus, Prdx6 possesses anti-fibrinolytic activity under inflammation by regulating autophagy.

P3783

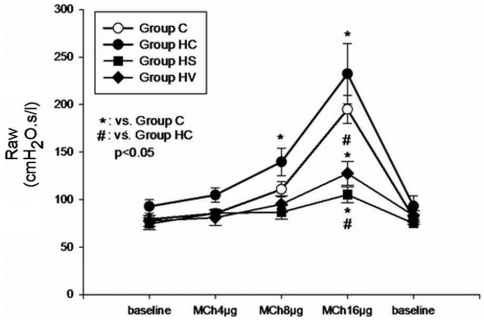
Prevention of hyperoxia-induced lung injury: Counterbalancing the deleterious effects of endothelin-1 in rat lungs

Dorottya Czövek[1], Yves Donati[2], Xavier Belin[1], Jean-Claude Pache[3], Ferenc Petak[4], Walid Habre[5]. *[1]Department of Anaesthesiology, Pharmacology and Intensive Care, University of Geneva, Switzerland; [2]Department of Pediatrics, Medical School, University of Geneva, Switzerland; [3]Department of Clinical Pathology, University of Geneva, Switzerland; [4]Department of Medical Physics and Informatics, University of Szeged, Hungary; [5]Pediatric Anesthesia Unit, Geneva Children's Hospital, University Hospitals, Geneva, Switzerland*

Rationale: Endothelin (ET-1) plays a major role in the hyperoxia-induced pulmonary hypertension leading to lung damage. We determined the role of the nitric oxide NO/ET-1 pathway in the lung function declineal changes following hyperoxia exposure in rats.

Methods: Airway resistance (Raw),respiratory tissue damping (G) and elastance (H) were obtained by forced oscillations at baseline condition and following incremental doses of iv methacholine (MCh) in 4 groups of 28-day-old rats. Animals were exposed for 3 days to: room air (Group C, n=6), hyperoxia (> 95% O2, Group HC, n=5), hyperoxia with concomitant administration of vasoactive intestinal peptide (VIP 150 μg/kg/day ip, Group HV, n=4) or oral sildenafil citrate (20 mg/day, Group HS, n=4).

Results: Hyperoxia led to significant increases in G (38.66%, 62.63%, 38.41% in groups HC, HV, HS respectively, $p<0.05$) and in H (58.91%, 67.3%. 70.85%, $p<0.05$) in all groups, while Raw did not change. Airway hyperresponsiveness to MCh was observed in rats of Group HC, which was prevented by treatments with VIP or sildenafil.



Conclusions: These findings evidence the beneficial role of NO and VIP pathways in preventing the lung inflammatory response to hyperoxia and indicating their protective potentials against the subsequent development of airway hyperresponsiveness.

Grant support: OTKA K81179. The first author is a laureate of the long-term ERS fellowship.

P3784

Protective effects of erythropoietin and N-acetyl cysteine on methotrexate-induced lung injury in rats

Hasan Kahraman[1], Mahmut Tokur[2], Ergul Kurutas[3], Selim Bozkurt[4], Vedat Bakan[5], Harun Ciralik[6], Nurhan Koksal[1]. *[1]Chest Disease, Kahramanmaras Sutcuimam University, Faculty of Medicine, Kahramanmaras, Turkey; [2]Chest Surgery, Kahramanmaras Sutcuimam University, Faculty of Medicine, Kahramanmaras, Turkey; [3]Biochemistry, Kahramanmaras Sutcuimam University, Faculty of Medicine, Kahramanmaras, Turkey; [4]Emergency Medicime, Kahramanmaras Sutcuimam University, Faculty of Medicine, Kahramanmaras, Turkey; [5]Pediadric Surgery, Kahramanmaras Sutcuimam University, Faculty of Medicine, Kahramanmaras, Turkey; [6]Pathology, Kahramanmaras Sutcuimam University, Faculty of Medicine, Kahramanmaras, Turkey*

Material and methods: Twenty-six female Sprague-Dawley rats were divided into 4 groups. Sham group was given subcutaneous (SC) injection of 0.3 ml of 0.9% NaCl (n= 5), MTX group was administered SC of 5 mg/kg methotrexate

(MTX), EPO group was administered, SC of 5 mg/kg MTX and 2000 IU/kg EPO, and NAC group was given 5 mg/kg MTX and 200 mg/kg NAC once daily for 4 consecutive days. At the fifth day, the right lungs were extracted. Oxidative damage was evaluated by measuring the malondialdehyde (MDA) level and superoxide dismutase (SOD) and catalase (CAT) activities. Histological damage was evaluated by inflammation and congestion scores.

Results: In MTX group MDA levels were significantly higher, CAT and SOD activities were significantly lower than those in the Sham, EPO and NAC groups ($P<0.05$). In EPO group MDA levels, CAT, and SOD activities were higher, but not significant than those in group NAC. In group MTX both scores were significantly higher than in group sham ($P<0.05$). The congestion score of group MTX was significantly higher than those of group EPO and NAC ($P<0.05$). When the group EPO was compared to the group NAC, the difference was not significant ($P>0.05$).

Conclusion: EPO and NAC have significant preventive effects on methotrexate-induced lung damage in rats.