Here we report that in HSD1-/- mice, lung permeability increases (6.1x10^-5 ±0.9x10^-5 v 10.7x10^-5 ±2x10^-5) 24hrs post-LPS. In addition, there was a build-up of apoptotic neutrophils (20.3x10^3 ±5.9x10^3 v 39.2x10^3 ±6.1x10^3), with a significant increase in the lung (33.2x10^4 ±5.7x10^4 v 81.8x10^4 ±1.4x10^5) 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 targeting 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
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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
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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 fibroblast treated with TGF-β. In this research we assessed the expression of Class I PI3K p110 isoforms in IPF lung tissue and cells derived from IPF 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 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 γ 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.

P3767
The role of pre-receptor glucocorticoid metabolism in regulating the severity of ALI
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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 current research suggests that local steroid metabolism by alveolar macrophages (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.
P3770
Inhibition of the sonic hedgehog pathway at the primary cilium prevents the effect of TGF-beta 1 on alveolar epithelial cells
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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 cell-fibroblast 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 with agonists (recombinant SHH, Smoothened agonist) or with inhibitors of the pathway (Cyclopamine, HPI-4, GANT61) in the absence or presence of TGF-beta1 (1-5ng/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/NIH3L abolishes the effect of TGF-beta 1 on the migration of epithelial cells but does not influence the effect of TGF-beta1 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-beta1 on A549 cells in vitro.

P3771
Protective effect of mesenchymal stem cells (MSC) on hypoxia-induced epithelial-mesenchymal transition of alveolar epithelial cells (AEC)
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Results: Reactive oxygen species (ROS) induced by hyperoxia have been shown to modulate epithelial-mesenchymal transition in vitro. In the present study, we investigated the protective role of mesenchymal stem cells (MSC) on the epithelial-mesenchymal transition (EMT) of adult human alveolar epithelial cells (AEC) induced by hyperoxia.

Methods: Rat AEC cultured on Transwell filters were exposed to HX (3 or 1.5% O2) in the presence of hyperoxia with antioxidants (vitamin E and C) or without. Cell viability, tight junction protein ZO-1 levels, expression of EMT markers (E-cadherin, ZO-1, α-smooth muscle actin) were assessed by ohm-meter, immunofluorescence and Western blot at 24h. ROS generation was measured in these conditions.

Results: The reductions of the TER in hyperoxic groups were associated with the reduction in the ZO-1 thickness and α-smooth muscle actin expression. In contrast, the antioxidant vitamins E and C had only a slight protective effect against hyperoxia damage.

Conclusion: Our results suggest that MSC may favor alveolar wound healing by preventing hyperoxia-induced EMT. We tested in vitro the paracrine effects of MSC on the phenotypic changes of AEC induced by HX.

P3772
Hyperoxia mediates barrier permeability dysfunction in 16HBE140 cells
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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 2.5×10⁴ cells/cm² in an air-liquid interface for 6 days where the transepithelial electrical resistance (TER) developed to 230±28 Ω cm². The experimental groups were divided into six sets, exposed for 24h to normoxia (21% O2, 5% CO2), hypoxia (95% O2, 5% CO2), hyperoxia with 10⁻³ M vitamin E, hyperoxia with 10⁻³ M vitamin C, hyperoxia with 10⁻³ M vitamin E and C, hyperoxia with 10⁻³ M vitamin E, hyperoxia with 10⁻³ M vitamin C and hyperoxia with a combination of vitamins E and C (10⁻³ M vitamin E and 10⁻³ M vitamin C). TER measurement, immunofluorescence staining of ZO-1 and RT-PCR to detect IL-8, IL-6, 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 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
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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 Respiratory were divided into 2 groups: long-term ICS group (ICS≥500/day futicasone propionate, ≥1 year) (n=21) and ICS naïve group (n=20). 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±1.71 vs 20.12±4.37 p≤0.019). The TLR2 intracellular expression (5.5±1.67 vs 12.81±4.89), the TLR2mRNAand TNFαmRNA expression also showed decreased trends in ICS long-term treatment group.

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.
P3775
The influence of roflumilast on the IV-type collagen level in patients with III stage COPD
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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 random 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 7.4 times higher than in almost healthy people, whose contents constituted (9.0±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
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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 in-vitro production 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 by 48.6 ± 0.3% (p < 0.05) and 64.5 ± 3.4% (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±2.4% (p < 0.05) times respectively. In comparison 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
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Cigarette smoke, which contains high concentrations of nicotine andndotoxin (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 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-vitro 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 FlexisVent® 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. Carbocarbol 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 TNF-α in vitro, mouse bronchial epithelial cells, but not in vivo. Combined treatment increased the production of MDA in the lungs remained high. In the 1st group of rats on the 3, 7 days logical changes the animals were divided into 2 groups. In both groups the level of MDA in the lungs remained high. In the 1st group the NBT values and PhA of AM were found to be increased, inflammation 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.

P3778
Alveolar macrophage activity in experimentally induced pneumonia
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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 transbronchic injection of pneumococcus culture. The animals were sacrificed at 1, 3, 7 day of PN course.

Results of the study: The development of PN in rats was accompanied by increase of MDA content in the lungs. The content of malonic dialdehyde (MDA) was determined in the animal lungs. The measurement of MDA in the lungs was performed by spectrophotometry.

Conclusion: The content of malonic dialdehyde (MDA) was determined in the animal lungs. The content of malonic dialdehyde (MDA) was determined in the animal lungs. The measurement of MDA in the lungs was performed by spectrophotometry.

P3779
Hydrogen gas alters the production of reactive oxygen species in alveolar epithelial cells in vitro
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Aim: The pulmonary toxicity of high concentration of oxygen during mechanical ventilation relates to reactive oxygen species (ROS). Hydrogen gas (H2) has potential as an alternative to various oxidant stresses on epithelial cells in vitro.

Methods: Human alveolar epithelial cells (A549) were incubated with amitcyclin which enhances the generation of superoxide anions (O2−) in mitochondria, with menadione which exogenously generates O2− and H2O2 in the cells, or with Cu+ (converted from Cu2+ by ascorbic acid) which exogenously generates hydroxyl radical by the Fenton reaction with added Cu2+ and endogenous H2O2. The viability of the cells as well as the levels of O2− and highly reactive ROS in the cells was evaluated with or without 2% H2.

Results: The viability of the cells incubated with menadione or Cu+ decreased or did not change in the presence of H2, respectively, while that with antimycin A probably due to the decrease in the production of O2− in mitochondria, while H2 cannot act protective against ROS induced by menadione or the Fenton reaction, meaning that H2 cannot overcome the effects of exogenously provided ROS.

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P3780

Effect of stress on free radical indices in bronchial asthma

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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 mM in patients with BA during stress and 4.5±0.05 mM 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 increased expression of lipid peroxidation, as evidenced by increased concentrations of MDA in EBC by 1.5 times in normal and 1.7 times in patients with BA.

Thus, the main increase 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

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VEGF exerts its biological effect through specific receptors, VEGFR-1 and VEGFR-2 and co-receptors, neuropilin-1 and neuropilin-2, leading to a complex regulatory system. 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 signal transduction of VEGF bioactivity.

A549 (ATCC) as a model for lung epithelial cells and Human pulmonary microvascular endothelial cells (HMVEC-1) were obtained and cultured in 24 hours in a PROX chamber in normoxic (N), hypoxic (95% O2, 5% N2), hypoxic conditions were represented by culture in the presence of Cobalt chloride (CoCl2).

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 VEGFR-1, NRP-2, however NRP-1 was significantly reduced compared to control values (p<0.05) in the presence of hypoxia and there was no significant trend to a reduction with both hypoxia and hyperoxia in VEGFR2. In HMVEC-1 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 hyperoxia.

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 plasmidin activator inhibitor 1 expression by regulating autophagy

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Objective: To evaluate the role of Peroxiredoxin6 (Prx6) in the expression of plasminogen activator inhibitor (PAI-1) in lipopolysaccharide (LPS) induced acute lung injury (ALI).

Methods and results: ALI was induced in Prx6(-/-) and C57BL/6J mice 4hrs or 24hrs after intratracheal instillation of LPS (5mg/kg), characterized by inflammation in higher wet/dry ratio, elevated protein concentration and increased neutrophils in bronchial/alveolar lavage fluid (BALF), which were more significantly in Prx6(-/-) mice. After LPS administration, PAI-1 mRNA expressions were markedly increased in a time-dependent manner and the PAI-1 concentration in BALF were markedly increased 4hrs and decreased nearly to baseline at 24hrs in Prx6(-/-) mice compared to C57BL/6J mice. Autophagy was significantly enhanced with higher expression of LC3B in Prx6(-/-) mice compared to C57BL/6J mice. The level of reactive oxygen species (ROS) in macrophages from Prx6(-/-) mice was significantly higher than that from C57BL/6J mice. The release of PAI-1 was significantly increased in macrophages from Prx6(-/-) 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 (MAPK) but not by c-Jun N-terminal kinase inhibitors.

Conclusions: In LPS-induced ALI, Prx6(-/-) mice increased PAI-1 expressions of partially dependent on enhanced autophagy in lungs and p38 MAPK and ERK in macrophages. Thus, Prx6 possess 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

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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) 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 conditions and following incremental doses of iv methacholine (MCh) in 4 groups of 28-day-old male 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. Hyperoxic hyperresponsiveness to MChs 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.

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P3784

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

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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 intravenously at 0.9% NaCl (n=5), MTX group was administered SC of 5 mg/kg methotrexate intravenously at 0.9% NaCl (n=5)

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(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.