410. Novel mechanisms in lung injury

P3894

Nitrosative and cytokine status in patients with COPD and chronic cerebrovascular diseases (CCVD)

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The aim of the study was to investigate the dynamics of nitric oxide (NO) stable metabolites concentration in exhaled breath condensate (EBC) as markers of the nitrosative stress and circulating inflammatory cytokines in COPD and CCVD.
Material and methods: 50 males inpatients were enrolled in the study (age = 51-67 y.o.). All patients were divided in two groups: group1 contains 23 patients with COPD exacerbation and CCVD; group2 contains 27 patients with COPD exacerbation only. The control group consisted of 21 healthy volunteers.The investigation of NO metabolism was performed by the estimation of total nitrates/nitrites (TNN) level and 3-nitrothyrozine (3-NT) concentration both in EBC and in blood plasma. The TNN concentration was measured using the spectrophotometric method; 3-NT and cytokines (TNF-α, IL-8) concentrations in blood plasma were investigated with specific enzyme immunoassay.
Results: The TNN levels in EBC as well as in blood plasma were significantly higher in group1&2 compared with control. The trend resulting to the increase of the TNN concentration in blood plasma was observed in patients with COPD and CCVD compared with COPD patients only (p=0.28). The 3-NT concentrations both in EBC and in blood plasma weren't changed in group1&2 compared with control. So, the statistically significant increase of inflammatory cytokines level in blood plasma compared with control was demonstrated (p<0,001). The cytokines activity in group1 was higher than in group2.
Conclusion: The results obtained demonstrate the increase of nitrosative/cytokines stress parameters as a systemic reaction in patients with COPD and CCVD compared with COPD patients only.

P3895

Leptin modulates host defense against chronic cigarette smoke inhalation in mice

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Rationale: Several hallmarks of COPD, including pulmonary and systemic inflammation, can be mimicked in mice by cigarette smoke (CS) exposure. We recently revealed increased expression of the pleiotropic adipokine leptin by resident lung cells in smokers and patients with COPD versus never-smokers. To unravel the involvement of leptin in COPD pathogenesis, innate and adaptive immune cell recruitment and remodelling upon chronic CS-exposure was evaluated in leptin-receptor deficient (*db/db*) mice.
Methods: WT C57/BKS and *db/db* mice were exposed to air or CS for 16 weeks (4 exposures/d, 5 d/wk). At 24h after the final exposure, bronchoalveolar lavage fluid (BALF) and lung tissue were processed to examine pulmonary inflammation and remodelling.
Results: CS exposure significantly increased leptin expression in bronchial epithelial cells and pneumocytes as compared to air-exposed WT mice (p<0.05). CS exposure resulted in accumulation of neutrophils, dendritic cells, macrophages and T-lymphocytes in BALF and lung tissue of both WT and *db/db* mice. However, CS-exposed *db/db* mice showed significantly higher number of neutrophils (p<0.05) and lower numbers of dendritic cells (DCs) and T-lymphocytes (p<0.05), compared to CS-exposed WT mice. In addition, chronic CS exposure resulted in increased hyaluronan deposition in the airway walls and development of pulmonary emphysema in WT mice and *db/db* mice, which was not different between both strains.
Conclusion: These data suggest a central role for the pleiotropic adipokine leptin in innate and adaptive immune cell accumulation after chronic CS inhalation in mice.
Funding: Weijerhorst Foundation, FWO Vlaanderen, Concerted Research Action of Ghent University.

P3896

Towards understanding the role of autoreactivity in COPD

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The role for autoimmunity in the pathogenesis of COPD is controversial, and the identity of putative autoantigens is subject to debate. In order to examine the role for autoantibodies in COPD, we cloned the complete variable light and heavy chains from single-cell sorted IgG or IgA-positive activated memory B cells isolated from COPD and control lung tissue, and expressed these as complete human Ig monoclonal antibodies (HumAbs). These HumAbs were tested for autoreactivity by histology using lung tissue sections of COPD patients and healthy controls and lung cell lines. In addition, serum of COPD patients and healthy controls was used to determine binding of antibodies to epithelial, fibroblast, and endothelial cell lines.
The HumAbs stained multiple cells and structures in lung tissue sections such as smooth muscles cells, epithelial cells, and the adventitia. The cell lines examined thus far, two epithelial cell lines, were also stained by the HumAbs.
Serum IgA and IgG antibody titers against epithelial, fibroblast, and endothelial lung cell lines overlapped considerably between patients and controls. As a consequence, the differences between titers of patients and controls were marginally significant. Interestingly, however, some COPD patients had a high serum antibody titer against one cell line, but a low titer against another cell line. Our results illustrate the complexity of autoreactivity in COPD in that concomitant with the preferential binding of different types of lung cells by antibodies in serum of COPD patients, individual antibodies may also target multiple cells and structures in the lung.

P3897

Paraoxonase activity in patients with COPD

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Aim: Oxidant/antioxidant disequilibrium is an important problem in pathogenesis of COPD. This disequilibrium is effective in development and progression of COPD. The increased oxidative stress in COPD is not only associated with rise of oxidants but also associated with the decrease of antioxidant capacity.
Paraoxonase 1 (PON1) functions as one of the endogen free radical clearing system in human body. PON1 is localized in clara cells, endotel cells and type 1 pneumocytes of the lungs.
In this study we aimed to study the PON1 activity in COPD patients with stable condition, had acute attack and developed respiratory failure.
Material and method: Twenty five patients with stable COPD (group1) (mean age 62,9±9,4), 25 cases with acute COPD attack (group2) (mean age 63,8±9,0), 25 patients with hypercapnic respiratory failure (group3) (mean age 65,0±12,9) and 25 healthy individuals for control group (mean age 34,8±9,8), totally 100 cases, were enrolled to the study. All cases enrolled to the study underwent routine biochemical analysis including PON1 activity and lipid profile.
Results: There was significant difference between groups with respect to PON1 levels (p<0.0001). PON1 activities of COPD patient groups (group 1=96,8±57,4U/L; group 2=51,4±32,8U/L; group 3=47,1±27,5U/L) were lower than control group (185,4±110,1U/L) (p<0.0001). Also PON1 activity of stable COPD patients was higher than the COPD cases admitted with acute attack or respiratory failure (group2 and 3) (p<0.05).
Conclusion: This findings show that PON1 activity may have a role in COPD pathogenesis and endogen antioxidants might be depleted by increased oxidative stress in COPD. This also advocates that oxidative stress may have a role in acute COPD attacks.

P3898

WITHDRAWN

P3899

Model of staged development chronic obstructive pulmonary disease (COPD) in rats

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Models of COPD open ways of studying pathogenesis, searching for therapeutic targets and new health care trends.
Aim: To reproduce the successive stages of COPD in experiment.
Methods: Model of COPD was induced in Wistar rats by long-time intermittent nitrogen dioxide (NO2) inhalation (15 ppm, 1,5 h/day for 60 days). Histological specimens were stained with hematoxylin-eosin. CD3 expression in bronchial walls and interstitium was determined by immunohistochemistry. TNFα and TGFβ were determined in serum and bronchoalveolar lavage fluid (BALF).
Results: After 15-day NO2 exposure acute response to injury was observed: epithelium desquamation and focal proliferation, swelling of submucosa, bronchial gland degeneration, lung tissue hyperextension were revealed. After 30 days cell infiltration of submucosa and hyperplasia of goblet cells were added. After 60-day exposure squamous metaplasia of ciliated epithelium, muscle plate atrophy, focal sclerosis, emphysema were identified. At this stage increase of CD3 expression was revealed in walls of bronchi and interstitium that indicated the presence of large number of T-lymphocytes (61,6±1,59 vs. 28,1±2,36 in control intact rats, $p<0,05$). TNFα increased in serum (125,9±16,21 vs. 60,9±6,34 pg/ml in control, $p<0,05$) and BALF (204,9±25,76 vs. 1,5±0,03 pg/ml in control, $p<0,05$). TGFβ increased in serum 18-fold and in BALF – 8-fold from control ($p<0,05$).
Conclusion: The model allows to reproduce stages of COPD from acute inflammation to lung tissue remoleling (emphysema and focal fibrosis). The model adequacy is confirmed by COPD symptoms: increased expression of CD3-lymphocytes in bronchial walls and lung parenchyma, multiple increased TNFα and growth factor TGFβ.

P3900

Lipoxin A4 receptor expression in smokers with and without COPD

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Background: The lipoxin A4 receptor, LXA4R/FPRL-1, is a G protein-coupled receptor. LXA4R with high affinity binds anti-inflammatory lipoxin LXA4 and with low affinity - N-formylated proinflammatory peptides. The aim of our study was to evaluate FPRL-1 expression in nonsmokers, asymptomatic smokers and in patients with COPD.
Patients and methods: 6 nonsmokers, 7 asymptomatic smokers and 5 moderate COPD patients undergoing lung resection for a solitary peripheral non-small cell carcinoma were enrolled in the study. Immunohistochemical methods were used to evaluate FPRL expression in airways and alveolar walls.
Results: FPRL-1 expression was observed in airways epithelial cells, macrophages, lymphocytes and neutrophils. Obtained results showed that asymptomatic smokers had increased numbers of FPRL-1 positive cells in alveolar walls compared to nonsmokers (157±59 vs 38±13 cells/mm^2, $p= 0.002$). At contrast, COPD patients had decreased numbers of FPRL-1 positive cells compared to asymptomatic smokers (23±7 vs 157±59 cells/mm^2, $p= 0.002$). In addition, COPD patients had a tendency of decreased FPRL-1 expression compared to nonsmokers. When all smokers were analyzed together, a significant positive correlation was found between the number of FPRL positive cells and airflow obstruction, FEV1% (Rho=+0.66, p=0.02).
Conclusion: Downregulated FPRL-1 in COPD patients may explain persistence of inflammatory process in alveolar area in COPD, whereas upregulation in asymptomatic smokers could serve as adaptive mechanism limiting inflammatory process.

P3901

Lung injury and apoptosis in COPD: Effect of a recombinant anti-protease derived from trappin-2

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Introduction: In chronic obstructive pulmonary disease (COPD) it is well established that neutrophil serine proteases: elastase (HNE), protease 3 (Pr3), cathepsin G (CG) contribute to lung injury. In order to target these proteases in COPD, we previously developed an anti-protease derived from trappin-2 modified to inhibit the three proteases at the same time. The goal of the study is to evaluate the protective effect of this inhibitor (trappin-2 A62L) against the degradation of lung epithelium and apoptosis of epithelial cells induced by proteases.
Methods: Alveolar epithelial cells (A549 cells) were exposed to serine proteases in presence or absence of trappin-2 A62L (T2A62L) with various concentrations and experimental conditions. The protective effects of T2A62L towards proteolytic damages were estimated by observation of changes in cell morphology and by the measurement of the cellular detachment. The protective effect of T2A62L towards A549 apoptosis induced by proteinases was evaluated by flow cytometry.
Results: Serine proteinases induce cell morphological changes, cellular detachment (35, 78, 85% for HNE, Pr3, CG respectively) and apoptosis of epithelial cells (5 and 2 fold increases with HNE and Pr3). Addition of T2A62L diminishes the proteolytic damages (5,10, 35% for HNE, Pr3, CG) and reduces significantly the rate of apoptotic cells.
Conclusion: The approach using a cellular model demonstrate that T2A62L exhibits anti-proteolytic and a protective effect towards apoptosis induced by serine neutrophil proteases. So, these results confirm the therapeutic potential of this inhibitor for treatment in COPD.

P3902

Effect of phototherapy in phospholipids' composition of membranes of lymphocytes in experimental COPD

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Introduction: Membrane phospholipids (PL) provide functional integrity of the cells of respiratory tract.
Aim: To study of membrane phospholipids' composition of peripheral blood lymphocytes in the dynamics of phototherapy in rats with experimental COPD.
Methods: An experimental COPD was reproduced in 60 white rats under the influence of tobacco smoke. For half of them we used the method of phototherapy of concentrated pulsed light (CPL) at wavelengths from 600 to 800 nm. Phototherapy was carried out daily for 10 days. The control group consisted of 10 healthy rats. To study the phospholipids' fractions of lymphocytes we used the high-flow horizontal chromatography.
Results: In rats with experimental COPD, compared with the control group, phosphatidylcholine (PC) and phosphatidylethanolamine (PE) were reduced by 31,8% ($P<0,01$) and 43% ($P<0,01$), the content of lysophosphatidylcholine (LPC) and lysophosphatidylethanolamine (LPE) was increased two-fold ($P<0,01$). Free fatty acids (FFA), compared with the control, increased by 47,2% ($P<0,01$) with a decrease in total phospholipids (TFL) by 17% ($P<0,01$). After 10 sessions of phototherapy we defined increase in PC and PE by 18,8% and 28,4% compared with the group of COPD without treatment, and this was accompanied by a decrease in LPC and LPE by 35,1% and 40,9% ($P<0,05$ in all cases). Tendency to normalization of FFA and TFL had no statistical significance.
Conclusion: Exprimental COPD in the rats is accompanied by quantitative changes in the main fractions of phospholipids in the membranes of peripheral blood lymphocytes. Conducting a course of phototherapy by CPL contributes to positive change in membrane phospholipids of these cells.

P3903

Possible role of 25-hydroxycholesterol on the pahogenesis of chronic obstructive pulmonary

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(Background) 25-Hydroxycholesterol (25-HC) is produced from cholesterol by cholesterol 25-hydroxylase and is related to atherosclerosis in vessels. Recently, 25-HC was reported to cause inflammation in various types of cells. The aim of this study is to assess the production of 25-HC in the airways of COPD and to elucidate the effects of 25-HC in neutrophil infiltration in the airways of chronic obstructive pulmonary disease (COPD).
(Methods) Eleven healthy never-smokers, 6 healthy ex-smokers, and 13 COPD patients took part in the lung tissue study. The expression of cholesterol 25-hydroxylase in the lung was investigated. Twelve healthy subjects and 17 COPD patients also took part in the sputum study. The amount of 25-HC in the sputum was also quantified. To elucidate the effects of 25-HC on neutrophil infiltration in the airways, 25-HC was intratracheally administered in mice.
(Results) The expression of cholesterol 25-hydroxylase was significantly enhanced in the lung tissue from COPD patients compared to healthy subjects. Cholesterol 25-hydroxylase was localized in alveolar macrophages and pneumocytes in COPD. The amounts of 25-HC in the sputum were significantly increased in COPD patients and the degree of 25-HC production was negatively correlated with the lung function. The amounts of 25-HC in the sputum had significant positive correlations with the interleukin-8 (IL-8) levels and neutrophil counts in the sputum. Treatment with 25-HC augmented neutrophil accumulation in the airways and the production of chemokines in mice.
(Conclusions) 25-HC production was enhanced in the airways of COPD patients and could cause neutrophilic inflammation.

P3904

Time course analysis of lung function and morphometric parameters in a murine model of emphysema

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Proteolytic enzymes have been used to induce emphysema in rodents to study mechanisms of this disease pathogenesis. However, few studies have evaluated the time course of the development of pulmonary emphysema after nasal instillation of elastase.
Objectives: To describe the progression of emphysema after porcine pancreatic elastase (PPE) nasal instillation in mice.
Methods: 64 adult male Balb/c mice received either a nasal drop of 50 μl (0.667 UI) of PPE (PPE) or saline (S) and were studied on days 1, 7, 14 and 21 after PPE instillation. For each time, we analyze airway resistance (RAW), tissue damping (Gtis) and tissue elastance (Htis). Inflammatory profile was performed in bronchoalveolar lavage (BAL) and both lungs were fixed with 10% buffered formalin infused through the trachea at 20cmH_2O for 24h and paraffin embedded for measurements of mean linear intercept (Lm).
Results: There was an increase in inflammatory cells in PPE groups since the 1st day, characterized by increase number of neutrophils and macrophages which remained until the 21th day. However the increase in lymphocytes became evident only at 7th day. We observed a decrease in Htis at 7th and 21th days, while there was an increase in Gtis at the 1st day and a decrease at the 21th day. We did not observe any differences for Raw values. The increase in Lm was observed in PPE group since the 1st day and was maintained throughout different times.
Conclusions: In this experimental model we observed an earlier inflammatory process concomitant with alveolar enlargement, suggesting that protease-antiprotease imbalance influence the development of emphysema.Supported by FAPESP, LIMHC-FMUSP, CNPq, Brazil

P3905

Effects of cancer cachexia on the alveolar morphology of the mouse lung

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Cancer cachexia is a complex syndrome with a significant reduction of body weight and a variety of systemic symptoms including respiratory dysfunction. In rodents, calorie restriction causes loss of alveolar surface area, the so-called nutritional emphysema. We hypothesized that alveolar alterations and loss of gas exchange surface area are present in the cancer-cachectic mouse.
C57Bl6 mice were randomly assigned to subcutaneous injection of Lewis lung carcinoma cells (tumor group, TG) or saline injection (control group, CG). Mice were sacrificed 21 days later and lungs were processed for light and electron microscopic design-based stereology (n=6 in each group) or for quantitative RT-PCR (n=5 each).
Body weight was reduced in TG vs. CG (TG: 17.4±0.4 g; CG: 22.1±1.0; $p<0.01$). Lung volume did not differ between TG (518.3±28.2 mm^3) and CG (468.3±50.5 mm^3). The alveolar surface area was similar in both groups (TG: 602.3±77.7 cm^2; CG: 496.2±58.2 cm^2). Although the total volume of lamellar bodies did not differ between the groups the volume of lamellar bodies per unit alveolar surface area was significantly reduced in TG (TG: 26.53±3.74 mm^3/m^2; CG: 40.25±13.05 mm^3/m^2; $p<0.05$). Quantitative expression of surfactant proteins A, B, C and D was not different between CG and TG as shown by RT-PCR.
In summary, despite a reduced ratio between the volume of the intracellular surfactant pool and the alveolar surface area in TG, there was no evidence for a significant disturbance of the gas exchange region due to cancer cachexia. In particular, weight loss was not associated with loss of alveolar surface area.

P3906

Aerobic training performed for short time do not decrease pulmonary allergic disease in mice

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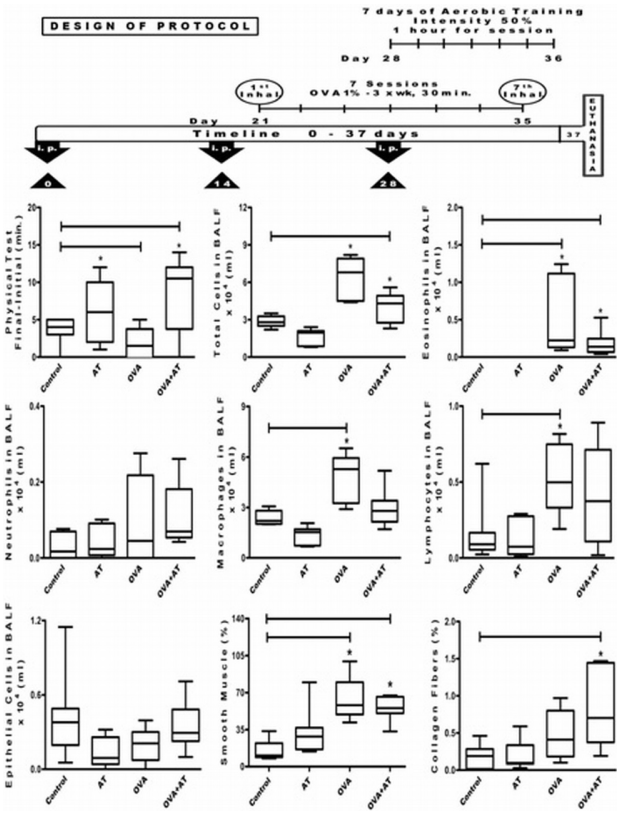
Background: Long term aerobic training (AT) seems to decrease airway inflammation in mice with pulmonary allergic disease.
Objective: Investigate the effect of short on AT in a murine model of asthma.
Methods: Male Balb/c mice were divided in 4 groups: Control, AT, ovalbumin sensitized (OVA) and OVA+AT. Sensitized groups received OVA i.p. (days 0,14, and 28) and were exposed to inhaled OVA1% (30min/3xweek) after the 21th day. The AT protocol as performed in a treadmill and began in the 28th day and was performed for 7 days (1hr/session, 50% of maximal intensity). Evaluations: In vivo respiratory mechanic (Flexivent), differential cell counting in the BALF, and collagen fibers depositions and smooth muscle thickness airways were evaluated by an image analyzer.
Results: Physical capacity was increased just in trained groups (AT and OVA+AT; $p<0.01$). No change was observed in respiratory mechanic among all groups ($p>0.05$); however, it was observed an increase in inflammatory cell migration and collagen fibers, and smooth muscle thickness in OVA and OVA+AT groups ($p<0.05$).



Conclusions: Short term aerobic training seems do not decrease features of pulmonary allergic disease in mice.

P3907

Contribution of TGFβ1 and TIMP2 to clinical activity of asthma and COPD

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Introduction: The process of bronchial tissue repair and remodeling in airway diseases depends on balance between production and degradation of different cytokines, leading to the regulation of extracellular matrix turnover finally.
Objectives: This study was designed to evaluate contribution of Transforming Growth Factor β1 (TGFβ1) and Tissue Inhibitor of Metaloproteinase-2 (TIMP2) to clinical activity and reversibility of asthma and chronic obstructive pulmonary disease (COPD).
Methods: In a cross sectional study on two groups of 29 asthmatics (14 males and 15 females) and 13 male COPD patients, we evaluated TIMP2 and TGFβ1 expression using semi-quantitative PCR on induced sputum samples. The relation among TIMP2 and TGFβ1 and pulmonary function test (PFT) indices and disease free period were assessed.
Results: Higher pulmonary function test (PFT) indices and longer disease free period was seen in COPD patients with raised expression of both TGFβ1 and TIMP2. On the other hand asthmatic patients had better pulmonary function status with raised TIMP2 and decreased TGFβ1 expression ($p<0.05$).
Conclusion: It seems that different effect of cytokines like TGFβ1 and TIMP2 in both diseases is depended on underlying inflammatory process in airways epithelium. We supposed that TGFβ1 bidirectionally affects activity of disease in asthma and COPD. Furthermore TGFβ1 as a biomarker in sputum may have a role for evidence-based drug prescribing like corticosteroids in patients with COPD and asthma.

P3908

The role of cathepsin D, H & K in the regulation of tumstatin levels in asthmatic airways

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Introduction: Angiogenesis is a prominent feature of remodelling in asthma. We previously reported that tumstatin, an endogenous angiogenic inhibitor which is the non-collagenous domain-1 (NC1) of the collagen IV α3 chain is absent from asthmatic airways. Tumstatin is released from the basement membrane by specific proteases. Cathepsins D, H and K (members of a broad family of proteases that degrade ECM proteins including collagen IV in other organs) are increased in inflammatory diseases and modulate tumour angiogenesis. We hypothesised that cathepsin D, H and/or K plays a role in the absence of tumstatin in asthmatic airways.
Methods: Cathepsin mRNA expression was measured by real time RT-PCR. Immunohistochemistry was used to measure cathepsin D, H and K in human airway tissue sections. Recombinant tumstatin and airway tissue sections were digested with active recombinant cathepsin D, H and K and the resultant cleavage products analysed by polyacrylamide gel electrophoresis.
Results: Human airway smooth muscle cells express cathepsin D and H mRNA. In both asthmatic and nonasthmatic airway sections inflammatory cells exhibit strong staining for cathepsin D. Cathepsin H and K are also strongly expressed in asthmatic airway tissues. Recombinant tumstatin was completely degraded by recombinant cathepsin D and H whilst cathepsin K degradation produced a 10kDa cleavage product. In human tissue sections recombinant cathepsin D completely digested tumstatin. Digestion with cathepsin K resulted in greater detection of the tumstatin antigen.
Conclusion: These findings suggest that cathepsin D, H and K may play a role in the regulation of tumstatin levels in the asthmatic airways.

P3909

Inhibitory profiles of alpha-1-antitrypsin from PiZ & PiSZ individuals and implications for tissue destruction in emphysema

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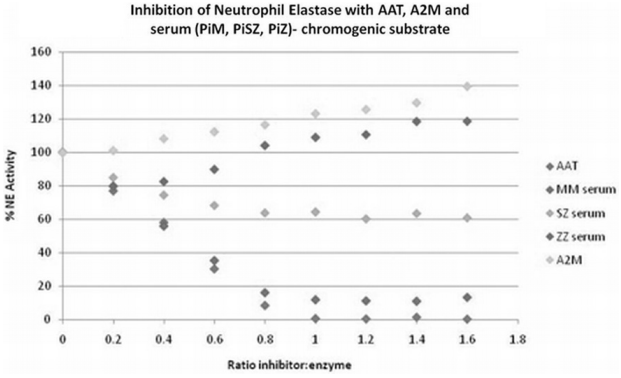
Introduction: Neutrophil elastase (NE) causes emphysema in animal models. Homozygote (ZZ) deficiency of its inhibitor alpha-1-antitrypsin (AAT) is associated with human emphysema. The role of heterozygote deficiency (SZ) is unclear.
Aims: To compare the inhibitory profiles of equimolar amounts of AAT from Z & SZ serum with pure AAT & M serum. The hypothesis is that Z AAT inhibits NE less efficiently than SZ & M AAT.
Methods: AAT concentration was measured in serum from Z, SZ & M patients. Increasing amounts of AAT were added to a fixed amount of NE. Residual NE activity was measured spectrophotometrically using both a chromogenic substrate and elastin. This was repeated with pure AAT & alpha-2-macroglobulin (A2M).
Results: With a low molecular weight chromogenic substrate, M serum AAT increasingly inhibited NE as the inhibitor:enzyme molar ratio increased to 1:1. Beyond 1:1 inhibition 15% residual NE activity remained, but not for pure AAT. For SZ serum residual activity was 60%. For Z serum and pure A2M enhanced NE activity was seen as inhibitor:enzyme ratio increased.



With elastin, inhibitory profiles of M, SZ & Z serum were similar to each other.
Conclusion: Enhanced NE activity with Z serum likely represents binding to A2M. Deficiency of AAT means that NE is more likely to bind to A2M. A2M:NE complexes retain proteolytic potential. These data may have implications for tissue destruction in emphysema.

P3910

Acute effects of an aerobic exercise session on airway inflammation in a murine asthma model

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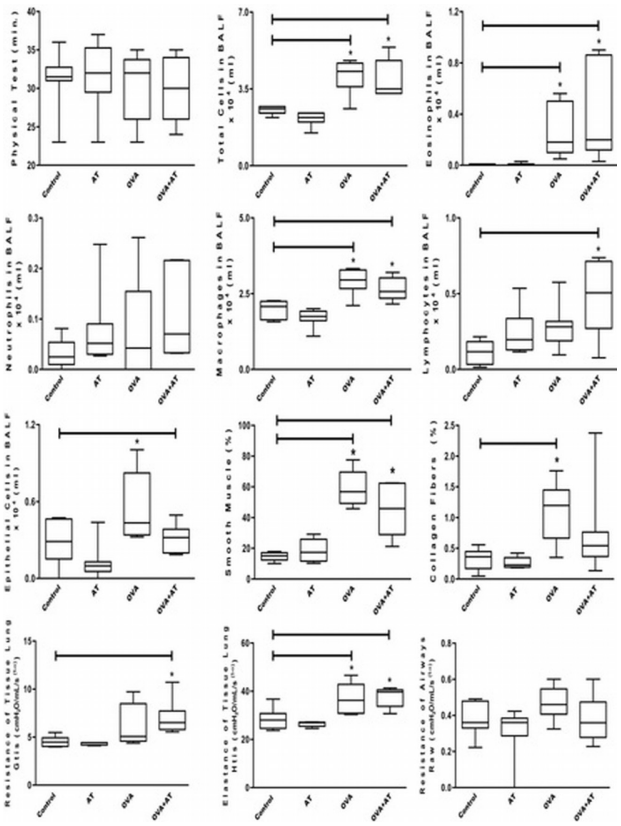
Background: Chronic effects of aerobic training (AT) seem to decrease inflammation in experimental asthma.



Objective: Investigate the effects of a unique exercise session of aerobic exercise in a murine asthma model.
Methods: Male Balb/c mice were divided in 4 groups: Control, AT, ovalbumin sensitized (OVA) and OVA+AT. OVA sensitization groups received i.p.(days 0,14,28) and OVA inhaled exposition (OVA1%, 3xweek/30min) after the 21st day. In the 28th day animals from AT and OVA+AT groups performed a session of treadmill running for 1 hour (50% maximal intensity). Evaluations: In vivo respiratory mechanic (Flexivent), different cell count in the BALF, and collagen fibers depositions and smooth muscle thickness in the airways were evaluated by an image analyzer.
Results: Initial physical capacity was similar among groups ($p>0.05$). BALF total and inflammatory cells, collagen fiber and the smooth muscle thickness were increased in sensitized groups ($p<0.05$). Parameters of respiratory mechanic (Gtis and This) were also increased in groups sensitized groups (OVA and OVA+AT; $p<0.05$).
Conclusions: Aerobic exercise when performed for acutely does not decrease features of experimental asthma such as cell migration, airway remodeling and respiratory mechanic.

P3911
High doses of N-acetylcysteine alone or in combination with inhaled corticosteroids and oxidative stress in patients with COPD
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Background: Oxidant/antioxidant interactions are known to be important processes in the pathogenesis of COPD. We aimed to evaluate the effects of 6-month oral N-acetylcysteine (NAC) treatment 600 mg twice daily alone or in combination with inhaled corticosteroids (ICS) on reactive oxygen species (ROS) production by granulocytes in peripheral blood measured by luminol-dependent chemiluminescence registration and its effect on pulmonary lipid peroxidation by malonyldialdehyde (MDA) level measurement.
Methods: 62 patients with stable COPD (36 males, mean age 66.8±7.5 years, GOLD stage I-IV) were divided into two treatment groups. Group 1 received bronchodilatators as basal treatment and NAC. Group 2 received NAC plus ICS in addition to basal treatment. Clinical examination, pulmonary function tests and blood collection were performed at baseline (T0) and repeated after 1 (T1), 3 (T3) and 6 months (T6) of treatment.
Results: Spontaneous ROS generation had trend to decrease at T3 in both groups, and achieved significant difference at T6 only in group 2 ($p=0.0004$). At the same time stimulated ROS generation did not significantly change in both groups ($p>0.05$). Antiperoxide serum activity was increased from T0 to T1 however further levels did not substantially changed. We registered MDA plasma level decrease in both groups during all treatment period, but significant difference from T0 to T6 was observed just in group 2 (1.8 μmol/L vs 1.4 μmol/L, $p=0.017$).
Conclusion: We conclude that combination of oral NAC 1200 mg/day with ICS for 6 months reduces the oxidant burden in airways of stable COPD patients and did not impact in patients treated with NAC alone.

P3912
Ceruloplasmine efficacy in patients with asthma exacerbations
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Ceruloplasmine (C) proves to be a valuable preparation in the treatment of patients with lung diseases because it is a copper containing enzyme which is considered to be the basic antioxidant factor.
The aim was to study the effect of of C on the production of active oxygen forms (AOF)) in patients with bronchial asthma (BA).
Methods: 40 patients with BA exacerbations were included in the study. The production of AOF in the blood of patients was studied by registration of luminol dependent spontaneous (SP) and staphylococcus activated (SA) chemiluminescence (ChL). The patients were divided into 2 groups. 20 patients received standard therapy with different basic medications. The other 20 patients with BA received combined therapy including C.
Results: In comparison with normal findings SPChL of blood in patients with BA was higher by 1,5-times and SAChL was higher by 1,8 times. It indicated increased production of AOF by blood cells. Treatment with C was associated with reduced generation of AOF in the blood, positive dynamics of clinical and laboratory findings. Patients who received standard therapy demonstrated symptoms of the disease and enhanced ChL intensity of the blood for a long time.
Conclusion: AOF production in patients with BA exacerbations was found to be increased The use of C reduces AOF production and improves the treatment efficacy.

P3913
Dynamics of oxidative stress parameters in exhaled breath condensate at controller treatment of bronchial asthma in patients with cold airway hyperpesponsiveness
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Background: Oxidative stress is an important factor of inflammation and airway hyperresponsiveness. Little is known about its dynamics during controller treatment of bronchial asthma (BA). Aim of the study was to evaluate the dynamics of oxidative stress products in exhaled breath condensate (EBC) after two-week controller treatment in BA patients with cold airway hyperresponsiveness (CAHR).
Methods: EBC from 49 BA patients with CAHR were studied before and after two-week controller treatment with inhaled glucocorticosteroids. The products of lipid peroxidation (diene conjugates (Å232), ketodiens conjugated with triglyceride (Å278)) were measured by spectrophotometric assay. Hydrogen peroxide (H_2O_2) was found out by electrochemical method with the help of biosensors on the basis of Prussian blue. The degree of cold bronchoconstriction was defined according to the fall of FEV_1 (ΔFEV_1) after 3-minute hyperventilation by cold (-20°C) air.
Results: After 2-week treatment high airway response to cold air hyperventilation (ΔFEV_1 -14.7±1.83% vs. -12.4±1.87%; $p>0.05$) was remained, but there was a significant decrease of H_2O_2 level from 1.10±0.07 till 0.95±0.06 nmole/ml ($p<0.01$), of diene conjugates from 0.48±0.02 till 0.43±0.01 E232/ml ($p<0.001$) and of ketodiens and conjugated triens from 0.09±0.01 till 0.08±0.01 Å278/ml ($p<0.001$) in EBC. The correlation was found between ΔFEV_1 and the level of H_2O_2 in EBC ($r=-0.37$; $p<0.01$) after the treatment.
Conclusion: The obtained data suggested the suppression of lipid peroxidation and damaging effect of airway cooling under inhaled glucocorticosteroids influence.