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342. Common and differing aspects of acute and chronic lung injury

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Proliferation studies in retinoic acid-induced alveolar regeneration

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Rationale: In emphysema the lung cannot spontaneously regenerate lost alveolar tissue. However studies have shown that treatment with retinoic acid (RA) promotes alveolar regeneration in rodent models of emphysema. We have previously shown that Dexamethasone (Dex) treatment of newborn mice inhibits secondary septation, causing a significant & permanent decrease in gas exchange surface area. Later RA treatment for 2 weeks results in restoration of alveolar surface area. We have used this model to study timing & cellular source of regeneration.

Method: Mice were Dex treated (0.4mg/kg, P4-P9 & P12-P16). At P42 animals received RA (2mg/kg) or vehicle (DMSO/oil) for 10 days. On P43 5 RA-treated & 5 control animals were given BrdU (100mg/kg i.p. injection) & killed 24 hrs later. This was repeated at 2-day intervals during RA treatment & 1 week after. Lungs were inflated fixed, volumes measured, & morphology assessed histologically by measuring alveolar Lm & surface area (SA). Anti-BrdU antibody labelled proliferating cells, & % labelled cell counts will determine proliferation rates at each sample time. Double labelling with cell-type markers will identify proliferating cells.

Results: Initial morphological results show no differences in Lm & SA between RA-treated & control animals.

Discussion: Morphological changes of regeneration are not detectable until a time point between P62 & P90 (sampling time in previous studies). Cellular response to RA may be more rapid, & further analysis will indicate the time course & identity of responding cells. These studies will determine if intrinsic lung cells are the source of RA-regenerated alveolar tissue.

P3706

Uric acid and bronchial oxidative stress in COPD

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Introduction: In the uric acid cellular synthesis are formed others pro-oxidative reactive substances as the super-oxide ion (O₂^{*}) which can react to the nitric oxide (NO) and form the peroxinitrites (ONOO). Both. O₂^{*} and peroxinitrites are responsible of denominated oxidative stress (OS).

Aims: To study if there is a relationship between uric acid and oxidative stress in sputum in patients with COPD.

Material and method: It was study a population of 51 patients diagnosed of COPD in stable phase (no exacerbation in the last six weeks) (FEV₁: 33.54 ± 4.14%).

To each one was measured uric acid analysis (enzymatic colorimetric method uricase) and malondialdehyde (MDA) (by mean of the reaction of thiobarbituric acid, TBARS) in induced sputum. The results are expressed in nmol/ mg prot. To evaluate the relationship between uric acid and MDA was made a correlation between both parameters by mean of Spearman test.

Results: When it was compared the uric acid and MDA levels in sputum was found a direct and significant relationship between both variables (r: 0.475; 95% confidence interval 0.229 – 0.66; p: 0.0004).

Conclusion: The uric acid in sputum in patients with COPD in stable phase is behaved as a marker of the local oxidative stress.

P3707

Relationship between proteases/anti-proteases system in induced sputum and spirometry testing in patients with chronic obstructive pulmonary disease

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The aim of this study was to assess the correlation between proteases/anti proteases system in induced sputum (IS) and spirometry testing and stages of illness in patients with COPD.

Method: Group of 43 COPD patients were analyzed. The mean age of patients, all males, was 54.6 ± 8.4. Mean FEV₁, mean FVC, mean SVC were 41.38% ± 18.45 58.22% ± 18.52, 53.51% ± 24.07, respectively. We investigated elastase, α₁-protease inhibitor, α₂-macroglobulin activity in an induced sputum.

Results: We found statistically significant positive correlation between elastase in IS and stages of COPD (r = 0.66, p<0.002). Activation of elastase was enlarged in 1,5; 2,0; 3,5 and 4,0 times, accordingly at I, II, III and IV stages of illness. Statistically significant negative correlation was between α₁-protease inhibitor, α₂-macroglobulin and stages of COPD (r = -0.70, p<0.001; r = -0.65, p<0.01, respectively).

Proteases/antiproteases in IS	FEV1	FVC	SVC
Elastase	r=-0,64 p=0,006	r=-0,64 p=0,006	r=-0,59 p=0,01
α1-protease inhibitor	r=0,67 p=0,003	r=0,61 p=0,009	r=0,62 p=0,008
α2-macroglobulin	r=0,70 p=0,002	r=0,72 p=0,001	r=0,72 p=0,001

Conclusion: the results of this study suggest that augmentation of stage COPD elastase activity grows and anti-proteases activity decreases.

P3708

Clearance of apoptotic cells in pulmonary tissue under the smoking influence

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Apoptosis and clearance of apoptotic cells are essential for tissue homeostasis and remodeling. Cell deletion by apoptosis leading to clearance by alveolar macrophages (AM) was investigated by cytological analysis of bronchoalveolar lavages (BAL) and blood samples of 9 nonsmokers and 6 smokers. Apoptotic parameters in BAL specimens stained by TUNEL and AM Acid Phosphatase (AcP) were evaluated by semiquantitative cytochemistry. Differential cell counting of BAL specimens was performed using light microscopy. Subsets of T cells were determined by fluorescent microscopy. There are significant increases of apoptotic index and apoptotic capacity and decrease of free apoptotic bodies in BAL of smokers in comparison with nonsmokers (p<0.05). Although there is no significant difference between BAL and blood T cell subsets in investigated groups, the regulatory pattern is changed in smokers. Mast cells exert regulatory influences on the process of ingestion of apoptotic cells by AM in nonsmokers and correlate inversely with CD4 and CD8 T cells in BAL of smokers. AcP in nonsmokers correlates with BAL macrophages (r=+0.85, p<0.05), but in smokers correlates inversely with BAL neutrophils (r=-0.84, p<0.05). As one of main remodeling molecules in pulmonary tissue, AcP is under regulatory influences of blood T cell subsets. There is significant correlation in nonsmokers between AcP and blood CD4 T cells (r=-0.78, p<0.05). It seems that remodeling of pulmonary tissue in situ under the smoking is influenced not only by local cell to cell interaction. Systemic regulatory influences on tissue remodeling molecules support an idea about dynamic compartmentalization of local immune response within the lungs of smokers.

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P3709**Analysis of the variation of antioxidant-related genes: glutathione transferases (M1, T1, P1), catalase, glutathione peroxidase 1 and NAD(P)H: quinone oxidoreductase-1 and their relationship to chronic obstructive pulmonary disease**

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Cigarette smoking is the major risk factor for developing COPD. Cigarette smoke contains massive amount of oxidants. Systemic and local increases in oxidants and decreases in antioxidants have been observed in smokers and individuals with COPD. Reactive oxygen species (ROS) are proposed to be a major cause of the cell and tissue damage. A few genetic variants, mostly in the forms of single nucleotide polymorphisms (SNP) were detected in genes that are directly implicated against oxidative stress.

We hypothesized that developing COPD may be associated with SNPs of antioxidant-related genes.

SNP and deletion variants of GSTM1 GSTT1, GSTP (Ile105Val), CAT1 (-262 C/T, 1167 C/T), GPX1 (Pro197Leu), NQO1 (609C/T) were investigated in COPD patient (N=320) and healthy individuals (N=422) from Ufa, Russia by PCR-RFLP method.

Analysis of the CAT gene variants(-262 C/T, 1167 C/T) revealed statistically significant differences in the haplotype frequency distributions between COPD and control group (X²=9.39 P=0.049). The patients with COPD showed elevated frequency of the CT-CC haplotype (22.41% vs 16.12%; X²=2.29 P=0.11). The genotype frequency distributions of the GSTP gene was significantly differed between COPD and control group (X²=4.62 P=0.05). But at the same time we did not find any differences in the genotype frequency distributions of the GSTM1 GSTT1 GPX1 and NQO1 genes within the patients and healthy groups.

Based on the reported data, it is concluded that CAT and GSTP1 genes variants probably play a substantial part in susceptibility to COPD.

P3710**Neutrophil accumulation without increased net gelatinolytic activity in the airways of tobacco smokers with stable chronic bronchitis**

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Studies of patients with exacerbations of COPD show an accumulation of neutrophils in the airways that is associated with a release of excess amounts of proteases including gelatinases. In this study on patients with stable chronic bronchitis, we determined net gelatinolytic activity and related it to neutrophil numbers locally in the airways.

Bronchoalveolar lavage fluid (BALF) was collected during a clinically stable phase in 29 smokers with a history of chronic bronchitis and recurrent exacerbations (CB). For comparison, we collected BALF in 8 asymptomatic smokers (AS) and 8 healthy never-smokers (NS) as well. BALF neutrophil counts were determined using light microscopy. The net gelatinase activity was determined using a gelatinase substrate assay and subsequent measurement of fluorescent intensity. The ratio between the net gelatinolytic activity and the neutrophil count was calculated.

Neutrophil counts (median [range]) were significantly higher in CB (188 [40-992], cells/mL) than in both AS (97 [35-211]) and NS (52 [10-165]), p<0.05 and p<0.01, respectively. The ratio of gelatinolytic activity per neutrophil (median [range]) was lower in both CB (2.3 [0.0-14.1]) and AS (2.3 [0.7-7.3]) than in NS (12.0 [0.0-61.4]) but this was not statistically significant.

In conclusion, despite considerable neutrophil accumulation in the airways in smokers with stable chronic bronchitis, the net gelatinolytic activity was not increased. This finding argues against an exaggerated neutrophil activity between exacerbations in chronic bronchitis, in line with our previous findings on neutrophil elastase and MMP-8.

P3711**Repeated exposure to cigarette smoke extract (CSE) in primary human lung fibroblasts - effects on proliferation rate**

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Background: We previously found that a single temporary exposure to CSE can induce a persistent reduction of proliferation rate and capacity in human lung fibroblasts.

Aim: To determine whether repeated vs single exposure to CSE has different effects and whether the effect depends on the time of exposure.

Methods: Using explant cultures from subpleural samples of lung resectates, fibroblasts were grown as subconfluent cultures. During quasi-linear growth, cells were exposed two times (early and late) to 2% CSE or DMEM for 2 days, with an

interval of 2 days in between. In duplicate experiments, cell number was monitored over time.

Results: Early, late and 2x exposure to CSE led to a significant reduction in final cell numbers which were 63, 52 and 40%, respectively, of control (n=15 and p<0.01 each, ANOVA). The 2x exposure to CSE had a greater effect than the single exposure (p<0.05). When expressing proliferation rate as the factor of increase after the second exposure, the single late exposure showed factor 1.33, which was different (p<0.05) from the time-matched factors after early, 2x and control exposure (3.15, 2.32 and 2.51, respectively).

Conclusions: The data indicate that the effect of a single temporary exposure to CSE on the proliferation rate of primary human lung fibroblasts is stronger if exposure occurs at a later stage of culture. Furthermore, a single early exposure does not prevent the effect of a second exposure. Assuming an inverse relationship between proliferation and cellular defense and its priming by the first exposure, this pattern appears to speak against purely oxidative damage exerted by CSE.

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P3712**KL-6 as a marker of alveolar inflammation in patients with ARDS**

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Introduction: KL-6, a marker of alveolar type II cells is elevated in plasma and epithelial lining fluid and may correlate with the severity of ARDS. The relationship between alveolar inflammation, infection and KL-6 has not been ascertained. We hypothesized that KL-6 in ARDS is dependent upon the severity of neutrophilic inflammation.

Method: Plasma and BAL samples were collected from 37 ARDS patients at day 1. Eitest-KL-6[®] elisa was used to measure KL-6. Bacterial growth in the BAL was determined quantitatively (>10⁴/ml).

Results: Plasma KL-6 were elevated in ARDS patients (548 U/ml compared to at risk (274, n=10, p=0.005) and normal controls(204, n=6, p=0.004). Plasma KL-6 correlated with the LIS (r = 0.4891, P=0.0021). In non-survivors plasma KL-6 increased significantly at day 4 (p=0.03) compared to day 1.

BAL KL-6 (589U/ml) was elevated in ARDS compared to normals(73.890, p=0.0003). BAL-KL-6 levels correlated with plasma KL-6 (r = 0.3260, p=0.04). BAL KL-6 also correlated with the BAL -MPO(myeloperoxidase) activity (r = 0.3417, p=0.044) and BAL cells/ml (r = 0.3991, p=0.0237).

BAL KL-6 of ARDS patients with significant pathogenic growth were similar (KL-6=659 U/ml, n=18) compared to those without significant infection (KL-6=481, n=24, p=0.246). Plasma results also did not show any difference.

Conclusion: BALF KL-6 is elevated in patients with ARDS and correlated with plasma KL-6. BALF KL-6 related to the severity of neutrophilic inflammation. Alveolar infection does not determine the KL-6 level in either BALF or plasma. KL-6 may represent a useful marker of alveolar type II cell dysfunction in ARDS as levels reflect the severity of lung injury and neutrophilic inflammation but not alveolar infection.

P3713**Moderate hypoxia coupled with hyperthermia as a trigger of alveolar macrophages apoptosis**

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Alveolar macrophages (AM) are important participators of lung defense system which constantly exposed to aggressive factors of ambient environment. The aim of this study was to examine the combined influence of hypoxia and heat on the AM viability. We revealed that 20h exposure of AM simultaneously to moderate hypoxia (10% O₂) and high temperature (42°C) resulted in significant increase (more than 3 times) of apoptotic cells. The abolishment of apoptotic AM was found when a potent antioxidant N-acetylcysteine (NAC) had been added in the medium before the incubation. Antihypoxic substance - succinate, in contrast, increased apoptosis more than 8 times and its action could be partially protected by NAC.

Surprisingly the more pronounced hypoxia (5% O₂) coupled with high temperature caused a less significant increase in the amount of annexin-V-positive cells in comparison with 10% hypoxia. Recently we have demonstrated that severe (5% O₂) but not mild (10% O₂) hypoxia stimulated NO generation by AM. We supposed that NO could be a factor that protects AM from hypoxia/hyperthermia-induced apoptosis. To examine whether NO would prevent cell death AM were exposed to the selective iNOS inhibitor, S,S'- (1,3-phenylenebis[1,2-ethanediy])bisisothiourea, during the hypoxia/hyperthermia incubation. But the result showed approximately 4-fold decrease of apoptotic AM level.

Summarising we concluded that hypoxia/hyperthermia exposure trigger apoptosis of AM that could be mediated by excessive production both reactive oxygen (ROS) and nitrogen species. NAC suppressed apoptosis decreasing ROS level, but succinate on the contrary intensified cell death probably by increasing ROS production.

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Three different agents cause similar acute responses after induced lung injury
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Lung injury induced by different agents cause a progressive breakdown of the lung extracellular matrix. It is well known that lung remodelling starts during first hours after lesion induction. We have performed a comparative study of early response after cigarette smoke exposure and administration of chemical agents like Elastase and Cadmium Chloride (CdCl₂). Matrix proteins, TGF-beta expression and NF-κB activation were analysed.

Materials and methods: Three experimental groups were developed. In the first group, Balb/c mice (n= 20) were exposed to sham or whole smoke from three consecutive Kentucky 2R1 cigarettes. In the second and third group, elastase and CdCl₂ were orotracheally administered to Wistar rats (n=48) respectively. 24 and 72 hours after administration, animals were sacrificed and lungs were severed. We analysed expression of matrix proteins (Collagen1, Collagen3, Elastin) and TGF-beta by Northern-Blot, and NF-κB activation by EMSA in first group.

Results: Matrix proteins, as well as TGF-beta expression, were decreased 24 hours after induced lesion in all groups in a similar manner. At 72 hours, all studied variables were increased in first and second group remaining near control values, but remain decreased in third group. NF-κB, analysed only in first group, showed an important increase at 72 hours.

Conclusion: Acute lung injury induced by different agents produce an early synthesis inhibition of matrix proteins and some mediators, that, at least, in animals cigarette smoke exposed, seems to be independent of NF-κB pathway.

P3715

Effect of simultaneous μ-opioidergic and adrenergic stimulation on the sheep parietal pleura

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ARDS is a clinical entity that often is complicated with pleural effusion. ARDS may originate from heroin abuse, and since it is a biologically stressful condition the plasma adrenaline levels are elevated. The objective of this study was to investigate the effect of simultaneous μ-opioidergic and adrenergic stimulation on the transmesothelial resistance (RTM) of the parietal pleura of sheep.

Intact sheets of parietal pleura were mounted in an Ussing chamber and measurements before and after the addition of morphine (10-9 M) and adrenaline (n=12) were conducted. The substances were placed both apically and basolaterally in all cases. The transmesothelial resistance is inversely correlated with the membrane permeability.

The control transmesothelial resistance (RTM) of the pleura was 20.12 ± 0.53 Ω*cm². This value increased significantly after the addition of the substances both apically and basolaterally (P<0.05).

It is known that both morphine and adrenaline increase the RTM of the parietal sheep pleura through distinct cellular paths. Our findings suggest that the combination of opioid and adrenergic stimulation leads to decreased permeability probably due to inhibition of Na⁺ transcellular transport and Na⁺-K⁺-ATPase.

P3716

Pleural fibrosis and thickening in a sheep empyema model leads to 21% decrease of pleural permeability

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The aim of the study was to investigate whether the mesothelial permeability of the sheep visceral pleura is altered after the incidence of fibrous adhesions and pleura thickening resulted by empyema.

Electrophysiological experiments were conducted on isolated sheep visceral pleura specimens of healthy controls and of sheep affected with Ovine Progressive Pneumonia (OPP) that results in empyema. The transmesothelial resistance measured is inversely correlated to the pleural permeability.

The transmesothelial resistance (RTM) of the visceral pleura of the control group was found to be 20.82 ± 0.42 Ω · cm² (n=74). The RTM of the visceral pleura of the group affected with OPP was found to be 25.27 ± 0.92 Ω · cm² (n=12). The transmesothelial resistance of the visceral pleura of sheep affected with OPP was found significantly higher than the control group.

Our results indicate that the electrophysiological profile of the visceral pleura mesothelium is altered after the occurrence of fibrous adhesions and thickening of the visceral pleura due to empyema. More specifically empyema leads to reduced pleural permeability.

P3717

Electrophysiological investigation of aquaporin-1 inhibition and adrenergic stimulation on the sheep parietal pleura

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The objective of this study was to investigate the inhibition of the water channel aquaporin-1 with adrenergic stimulation on the electrophysiological profile of the parietal pleura of sheep. Aquaporin-1 is a water channel with partial cation transporting properties and has been found to be expressed on the parietal pleura. Intact sheets of parietal pleura were mounted in an Ussing chamber and measurements before and after the addition of the aquaporin-1 inhibitor, HgCl₂ (10-4 M) (n=12), adrenaline (10-7 M) (n=12), and combination of HgCl₂ and adrenaline (n=12) were conducted. The substances were placed both apically and basolaterally in all cases. The transmesothelial resistance is inversely correlated with the membrane permeability.

The control transmesothelial resistance (RTM) of the pleura was 20.44 ± 0.78 Ω*cm². This value decreased significantly (P<0.05) after the inhibition of aquaporin-1 apically, increased after adrenaline addition (P<0.05) and increased significantly after the combination of aquaporin-1 inhibition and adrenergic stimulation apically (P<0.05).

Our findings suggest that inhibition of aquaporin-1 leads to increased permeability probably due to induction of other ionic channels, and the combination with adrenergic stimulation leads to partial inhibition of adrenaline's effect.

P3718

Comparison of *in vivo* and *in vitro* inflammatory potential of inhalable cosmetics

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This project aims to develop *in vitro* assays to predict the inflammatory potential of inhalable cosmetic ingredients. Organic polymers S2218600, S2429901 and S2219200 (referred to as P1, P2 and P3, respectively) of varying toxic potential, designed for use in hairsprays, were used as model substances. *In vivo* inflammogenic potential was evaluated by assessment of inflammatory cell profile (macrophage (AM), neutrophil (PMN)) of broncho-alveolar lavage fluid (BAL) 24hrs after polymer instillation in Sprague Dawley rats (single instillation 0.5mg or 2mg). In addition, cultured NR8383 AM-like cells were administered polymer in 0 or 2% FCS conditions for 24h and supernatants analysed for indicators of cytotoxicity and inflammatory mediator release.

The *in vivo* studies showed that both P1 and P2 caused significant PMN influx into BAL (data = mean ± sem PMN (x10⁶): control 0.31±0.15; P1 19.92±5.76; P2 16.80±5.06; P<0.05 vs control). P2 caused a significant increase in AM number in BAL (data = mean ± sem AM (x10⁶): control 4.49±0.67; P2 9.87±3.16; P<0.001 vs control). P3 had no effect on BAL cell profile. BAL TNFα levels were significantly increased following instillation of P2. Thus the 3 polymers were ranked for potential to cause pulmonary inflammation: P2<P1<P3.

In vitro studies showed that the polymers could be ranked similarly for cytotoxic potential and ability to stimulate release of TNFα (data = mean ± sem TNFα (pg/mL) following treatment with 5μg/cm²: P2 119±68; P1 44±22; P3 0±3.47; P<0.05 P2 vs control). The similarity of hazard ranking for *in vivo* inflammation and toxicity to macrophages *in vitro* suggests that the *in vitro* approach may prove useful in testing such materials.

P3719

IL-8 and VEGF levels in the bronchoalveolar lavage (BAL) of rabbits submitted to pleurodesis with talc of different size particles

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Purpose: Acute respiratory failure is considered the most serious complication in talc induced pleurodesis. The pathophysiology is still unclear, and probably involves the inflammatory systemic response associated with talc particles dissemination.

Methods: Fifty rabbits received talc (400mg/kg) into the right pleural space. Rabbits were divided in 2 groups: small talc (ST, D50=6.4 mm) and mixed talc (MT, D50=21.2mm) and 5 animals were used as control (no injection). After 6, 24, 48, 72 or 96h the rabbits were sacrificed and the right (R) and left (L) BAL samples were collected from both lungs and processed for IL-8 and VEGF measurements. Statistics: ANOVA and t-test.

Results: IL-8 and VEGF levels were significantly higher than control for RBAL and LBAL in all groups (p<0.05). In the comparison between both types of talc,

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IL-8 was higher at all times for ST ($p < 0.05$). In addition, the RBAL presented higher IL-8 levels than LBAL. For both sides, VEGF levels peaked at 48h in MT group, and were steadily high at all times in ST group. VEGF concentration was higher in the RBAL than LBAL ($p < 0.05$) with no difference between both talc groups.

Conclusion: High levels of IL8 and VEGF in BAL in talc injected rabbits suggest an important participation of these cytokines in the acute pulmonary inflammatory response. The increased IL8 in RBAL and in LBAL observed with ST indicates a more pronounced inflammatory response to this agent, reinforcing the role of the smaller particles in the systemic response to talc.

P3720**The effect of combined hypoxia and hyperthermia on the metabolism of alveolar macrophages**

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It is well known that acute lung injury may result in reduced oxygen tension around alveolar macrophages (AM) in the affected lung areas. The exposure of AM to hypoxia could perturb their functions and therefore trigger the progression of certain lung injury. There is evidence that the effect of hypoxia on cells is mediated by the cytokine secretion which could change after the exposure to heat. So combined effect of hypoxia and high temperature, which is the most frequent symptom accompanied to hypoxia, on metabolic and functional activity of AM was studied in this work.

It was shown an approximately 2-fold decrease of phagocytic activity of AM exposed to 5% O₂ and 42°C for 2 hours in comparison with control cells. Simultaneously was observed significantly increased activity (on 55%) of lactate dehydrogenase and decreased (on 23%) – of succinate dehydrogenase. Sharp disorders in oxygen/antioxygen balance were also revealed in AM after hypoxic/hyperthermic influence. It revealed in graded increase of H₂O₂ and NO concentration as well as TBARS level and parallel attenuation of catalase and glutathione peroxidase but not superoxide dismutase activity. Finally was found a marked augmentation in number of the apoptotic AM at the low O₂ concentration and the high temperature in comparison with control value.

All these disturbances occur only slightly when 10⁻⁵M N-acetylcysteine was added in the incubation medium before the treatment. These observations suggest that oxidant/antioxidant disbalance is the early steps of hypoxia/hyperthermia-induced disorders in AM and these disturbances could be prevented by using the compounds with antioxidant activity.

P3721**The use of N-acetylcysteine in animal models of hepatopulmonary syndrome**

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AIM - to investigate the potential antioxidant of N-acetylcysteine (NAC) in the hepatopulmonary syndrome, in the biliary cirrhosis through bile duct ligation in rats. **METHODS** - Male Wistar rats were used and the hepatic integrity was investigated through blood enzymes, arterial blood gases and oxygen saturation, together with the oxidative damage (thiobarbituric acid reactive substances – TBARS), antioxidant enzyme (superoxide dismutase – SOD, nitrates (μmol/L) and histology of the lung. The animals were divided into 4 groups: CO - in which the surgery of the biliary duct main ligation was simulated; LDB - in which the surgery of the biliary duct main was accomplished; CO + NAC - in which the surgery of the biliary duct main ligation was simulated received treatment with NAC after the 14th day of the surgery; LDB + NAC - in which the surgery of the biliary duct main was accomplished and received treatment with NAC after the 14th day of the surgery. The NAC was administered by intraperitoneal via, in a concentration of 10mg/Kg, for 14 days. **RESULTS** - improvement in the enzymatic parameters and arterial blood gases after the treatment with NAC. Reduction of the oxidative damage was verified through TBARS, as well as the antioxidant enzymes SOD that are shown with close values to those of the group controls after the administration of NAC. The histological analysis, has shown vasodilatation in the lung, those phenomena were reverted after the use of NAC. **CONCLUSION** - the treatment with N-acetylcysteine prevents the development of hepatopulmonary syndrome in this model.

P3722**The histopathological effects of normo- and hyperbaric oxygen therapies on lung and brain tissues in rats exposed to acute carbon monoxide**

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Carbon monoxide (CO) poisoning remains a serious public health problem. Normo- and hyperbaric oxygen (O₂) treatment strategies are applied in CO poisoning caus-

ing diffuse tissue hypoxia. The aim of our study is to evaluate the histopathological changes of normo- and hyperbaric O₂ treatments on lung and brain tissues that are exposed to CO. Twenty six, young, female, healthy Sprague-Dawley rats weighing 200-250 g each were grouped into four. All groups were exposed to 99.5% CO for 2 minutes. Different treatment protocols were applied. The first group consisted of 6 rats exposed to CO that were later left at room air for 30 minutes. The second group consisted of 6 rats that received 100% normobaric O₂ therapy for 30 min. The third group of 6 rats received 2.4 atm absolute (ATA) hyperbaric O₂ treatment for 30 min. and the fourth group of 8 rats received acetylcysteine injected intraperitoneally followed by hyperbaric O₂ therapy for 30 min. Carboxihemoglobin (COHb) level was calculated for justification of CO poisoning in rats. Lung and brain tissue samples were taken from the rats at the end of treatments and histopathologically evaluated. In our study, perivascular edema, congestion and alveolar hemorrhage, septal thickening were evaluated for lung pathology and hydropic degeneration, parenchymal edema, vascular congestion and perivascular edema for brain pathology. Data were calculated using ki-square for statistical significance. We conclude that hyperbaric O₂ and hyperbaric O₂ combined with N-acetylcysteine increase ischemic reperfusion phenomenon significantly in rats exposed to acute CO poisoning.

P3723**Approach to pathogenetic treatment of experimental pulmonary fibrosis**

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The suppression of fibrotic process may be attained due the prevention of excessive production of reactive oxygen species (ROS) by activated inflammatory cells. The anticytokine (interleukin-1 receptor antagonist, IL-1ra) and complex of antioxidants (AO) were chose as possible fibrogenesis modulators.

The experimental model of toxic fibrosis alveolitis was induced by intratracheal bleomycin (BL) administration (10 mg/kg) in rats. For 14 days before lung injury by BL rats received AO per os, and IL-1ra (150 mg) was instilled via trachea in 5 min later BL. Control rats received 0.9% NaCl instead of AO and IL-1ra. Bronchoalveolar lavage (BAL) fluid was obtained on 3, 14, 30 days post BL. The alveolar macrophage (AM) production of ROS was determined by luminol-dependent chemiluminescence (CL). In control rats AM oxidative activity exceeded normal level through 30 days after BLM: peak CL was 23,0±2.2; 12,6±1.3 and 9,0±0.7 mV respectively at 3, 14 and 30 day post BL ($p < 0.05$, normal level 4,6±0.7 mV). In rats treated with AO+IL-1ra respective peak values of CL were 8,6±0.9; 4,9±0.6 and 4,4±0.5 mV ($p < 0.05$ in comparison with control), i.e., ROS production didn't differ from normal level ($p > 0.05$). On 14 day post BL (acute inflammatory phase) BAL fluid of treated rats contained less neutrophils (21,2±1,9%) than in control (66,2±4,5%, $p < 0.05$). Morphologic investigation showed the decrease of inflammatory and fibrotic manifestations in treated rats in comparison with control ones.

Conclusion. Treatment of experimental fibrosis alveolitis with IL-1ra and AO complex reduced ROS generation by AMs, decreased neutrophil influx and inflammatory reaction, prevented the development of BL-induced oxidative stress in lungs.