

SUNDAY, SEPTEMBER 25TH 2011

71. Recent developments in COPD

P414

LSC 2011 Abstract: The role of lipoxin A4 in the chronic obstructive pulmonary disease

Liga Balode, Gunta Strazda, Normunds Jurka, Uldis Kopeika, Agnese Kisliņa, Marina Beinare, Maris Bukovskis, Immanuels Taivans. *Faculty of Medicine, University of Latvia, Riga, LV Institute of Experimental and Clinical Medicine, University of Latvia, Riga, LV*

The aim: Chronic obstructive pulmonary disease (COPD) is characterized by persistent inflammatory reaction with a dominance of neutrophil involvement. It is established, that during a healing of acute inflammation, a switching of arachidonic acid (AA) metabolism from leukotriene (Lt) to lipoxin (LX) production occurs. Therefore we hypothesized that in COPD patients the lipoxin production could be insufficient. Hence we measured the content of LXA₄ and LtB₄ in induced sputum supernatant (IS) in COPD patients and healthy subjects.

Materials and methods: 17 COPD patients and 7 healthy persons were studied. The age and gender ratios were similar in both groups. Sputum induction was performed according to the ERS protocol. LXA₄ and LtB₄ content in sputum supernatant was assessed by ELISA.

Results: COPD patients had decreased concentration of LXA₄ in induced sputum compared to healthy persons (0,514 ng/ml and 1,310 ng/ml, respectively, $p=0,00784$).

LtB₄ content in induced sputum did not significantly differ between COPD group and healthy persons group (3,551 ng/ml and 3,754 ng/ml, respectively). The ratio LtB₄/LXA₄ in COPD patients was three times higher compared to healthy persons (ie. 9,816 ng/ml and 3,425 ng/ml; $p=0,00982$).

Conclusions: We concluded that the chronic obstructive lung disease is characterized by suppressed production of lipoxins. This insufficiency may be responsible for a persistence of neutrophilic inflammation in airways.

P415

LSC 2011 Abstract: Inflammation and COPD: Protective effect of the recombinant anti-protease trappin-2 A62L, on lung epithelium

Annabelle Tanga, Alhame Saidi, Sandrine Dallet-Choisy, Marie-Louise Zani, Thierry Moreau. *Inserm U618 Protéases et Vectorisation Pulmonaires, Université François Rabelais, Tours, FR*

Introduction: Inflammation in chronic obstructive pulmonary diseases (COPD) results in a protease/anti-protease imbalance that leads to a massive release of neutrophil serine proteases (elastase, proteinase 3 and cathepsin G). These proteases stimulate secretion of mucus and pro-inflammatory cytokines. In order to target serine proteases, we designed a recombinant inhibitor derived from trappin-2 which is able to inhibit all three proteases at the same time. The aim of the study was to evaluate the inhibitory, anti-inflammatory and anti-secretory effects of trappin-2 A62L (T2A62L) on lung epithelium exposed to neutrophil serine proteases.

Methods: A549 cells were exposed to proteases for 24H with or without addition of T2A62L. Protective effect of T2A62L towards the degradation of cell junctions by proteases was analyzed by immunofluorescence. Levels of mucus secretion were determined by measuring the rate of expression of mucin genes and the anti-inflammatory activity of T2A62L was investigated by measuring the rate of pro-inflammatory mediators release after LPS stimulation.

Results: Neutrophil serine proteases proteolytically degrade cellular junctions and increase mucin gene expression. T2A62L added to the culture medium inhibits the degradation of cell junctions proteins (E-cadherin, ZO-1), decreases MUC5AC and MUC5B mRNA expression induced by elastase and IL-6 and IL-8 productions.

Conclusion: Our results demonstrate that T2A62L exhibits anti-proteolytic, anti-inflammatory and anti-secretory effects. This new, anti-protease may therefore be of therapeutic value in treating inflammatory lung diseases such as COPD.

P416

Plant proteinase inhibitor from *enterolobium contortisiliquum* (*EcTI*) attenuates elastase-induced pulmonary inflammatory and remodeling alterations in mice

Osmar A. Theodoro Jr.¹, Rafael Almeida-Reis¹, Leandro V. Oliva¹, Bruno T. Martins-Oliveira¹, Daniel S.S. Mello¹, Fernanda D.T.Q.S. Lopes¹, Carla M. Prado³, Patrícia M.G. Paiva², Edna A. Leick¹, Marlon V. Brito², Milton M. Martins¹, Maria L.V. Oliva², Iolanda F.L.C. Tibério¹. ¹Clinical Medicine, Faculty of Medicine, University of Sao Paulo, Sao Paulo, Brazil; ²Biochemistry, Federal University of Sao Paulo, Sao Paulo, Brazil; ³Biological Sciences, Federal University of Sao Paulo, Diadema, Brazil

Aims: To evaluate if a plant Kunitz proteinase inhibitor *EcTI* contributes to inactivation of elastase-induced mechanical, inflammatory and extracellular matrix remodelling alterations.

Methods: C57Bl6 mice received elastase (50ml/animal- IN-E group). Control group received saline (Ve group). Mice were treated with *EcTI* (2mg/kg) at days 1, 7, 14, 21, 28 and 35 after elastase instillation (I-E group). At day 40, mice were anesthetized, mechanically ventilated and we analyzed respiratory system resistance and elastance, tissue elastance, tissue damping, and airway resistance. Afterwards, BAL was performed and lungs were removed. By morphometry, we quantified the mean linear intercept (Lm), collagen and elastic fibers in distal lung. **Results:** There were no differences in pulmonary mechanics comparing all groups. In E-group, there was an increase in BAL-total cells, BAL-lymphocytes, BAL-neutrophils, collagen, elastic fibers and LM compared to control ($p<0.05$). In I-E group, *EcTi* attenuated the increase in BAL-total cells ($65.2\pm 3.3\times 10^4$ cells/mL) and BAL-neutrophils ($1.3\pm 0.7\times 10^4$ cells/mL) compared to E-group (respectively: $110.7\pm 9.7\times 10^4$ cells/mL and $8.0\pm 1.4\times 10^4$ cells/mL, $p<0.05$). There was a decrease in the collagen content in I-E group ($43.2\pm 4.3\%$) compared to E-group (59 ± 2.5 , $p<0.05$).

Conclusions: This proteinase inhibitor (*EcTI*) reduced elastase-induced pulmonary inflammatory and extracellular matrix remodeling alterations induced by elastase. Although more studies need to be performed, this inhibitor may contribute as potential therapeutic tool for COPD management.

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P417

Impaired respiratory function in mice exposed to cigarette smoke

Sofia Jonasson¹, Bo Koch¹, Linda Elfsmark¹, Christine Akfur¹, Anders Buch^{1,2}. ¹Swedish Defense Research Agency, FOI, Division of CBRN Security and Defense, Umeå, Sweden; ²Department of Public Health and Clinical Medicine, Umeå University, Umeå, Sweden

Introduction: We have aimed to develop a mouse model of cigarette smoke (CS)-induced small airway disease and emphysema. This model will be used for studies of pathogenic responses in the development of emphysema and for preclinical evaluation of new drug candidates.

Methods: BALB/c mice were exposed to CS (nose-only), 5 days a week during 10 weeks (Gr 1: 6 cigarettes during 10 min, twice a day; Gr 2: 12 cigarettes once a day during 20 min; Gr 3: 12 cigarettes during 20 min, twice a day; Gr 4: control, room air). The experiment ended on day 71-73 and day 85-87 (two additional recovery weeks without CS smoke) with thoroughly investigated lung mechanics (FlexiVent), inflammatory cell counts in bronchoalveolar lavage (BAL) and lung histology.

Results: CS induced an increased respiratory resistance ($p<0.05$), tissue resistance ($p<0.05$), tissue elastance ($p<0.05$), and decreased respiratory compliance ($p<0.05$), in all CS groups compared to control groups. However, there were no differences in lung mechanics and total cell counts in BAL within the CS-groups. After the recovery weeks, the CS-induced effects in the lung were sustained in all groups except for Gr 2. Lung histology showed no obvious alveolar damage in peripheral airways.

Conclusion: In our mouse model there is a marked effect on lung mechanics, suggesting that CS induce small airway inflammation which is not dose dependent. Our result indicates that it is the exposure time, rather than the number of cigarettes, that determines the detrimental effects in the mouse lung. Additionally, this model can be used for combined exposure of allergens and air-borne particulate pollution in order to investigate aggravating effects of inhaled particles on emphysema and COPD.

P418

Different effects of mesenchymal stem cells on elastase-induced pulmonary emphysema and macrophages activity in rats

Alexander Averyanov¹, Anatoly Konoplyannikov², Andrey Chernyaev³, Vasily Petrov², Oleg Kuzovlev¹, Andrey Bruhovetsky², Olga Konoplyannikova², Ekaterina Agaeva², Natalia Kulagina¹. ¹Clinical Hospital No. 83, Federal Medical and Biologic Agency, Moscow, Russian Federation; ²Cell Therapy Department, Medical Radiological Scientific Center, Obninsk, Russian Federation; ³Morphology Department, Pulmonology Research Institute, Moscow, Russian Federation

The aim of the study was to assess lung tissue changes and macrophages activity (MA) after mesenchymal stem cells (MSCs) transplantation in elastase model of pulmonary emphysema in rats.

Methods: Forty 3-months old Wistar rats were randomized into 4 groups. Control group (group 1) was injected intratracheally 0,4 ml of normal saline, other animals (groups 2-4) received one intratracheal injection of 20 units (U) porcine pancreatic elastase in 0,4 ml of saline. Next day (group 2) and 7 day (group 4) rats were intravenously injected 2000000 autologous MSCs in 0,5 ml of saline. Group 4 was used as emphysema control. Before killing on 21-st day rats were undergone peritoneal lavage with analysis of hemi-luminescent macrophages activity in the obtained fluid

Results: The lungs of groups 2-4 had various degrees of centriacinar emphysema. There were no significant differences between the sizes of dilated respiratory bronchioles in groups 2 & 4. Only in the 2-nd group we observed the lymphoid infiltration around arterioles. The width of respiratory bronchioles in group 3 was on 41% less than in the 4-th. The Index of hemi-luminescent MA has made 28,8±1,1 U (1 group), 35,8±1,6 U (2 group), 31,9±1,9 U (group 3), 57,3±1,3 U (group 4). **Conclusions:** MSCs have various effects on damaged lung tissue at different periods of transplantation. MSCs can enhance the local vascular inflammatory response in the early period of elastase injury. More later injection of MSCs can repair the structure of acini in acute model of emphysema in rats.

P419

IL-10 resolves the neutrophilic inflammation in mice exposed to cigarette smoke

Manabu Higaki¹, Hiroo Wada¹, Tetsuo Yasutake¹, Shinichiro Mikura¹, Kojiro Honda¹, Masuo Nakamura¹, Mamoru Niikura², Fumie Kobayashi², Shigeru Kamiya², Hajime Goto¹. ¹Department of Respiratory Medicine, Kyorin University School of Medicine, Tokyo, Japan; ²Department of Infectious Diseases, Kyorin University School of Medicine, Tokyo, Japan

Introduction: IL-10 plays a suppressive role in the inflammation. In order to elucidate the role of IL-10 in the inflammation caused by cigarette smoke, mice exposed to cigarette smoke were investigated.

Materials & methods: Mice (C57BL/6J) were exposed either to cigarette smoke or to environmental air for 5, 8 or 12 days. To characterize the inflammation, cellularity in bronchoalveolar lavage (BAL) fluid was investigated. The levels of inflammation-associated cytokines, such as IL-10, KC, MIP-2, TNF-α and GM-CSF, were further determined by measuring both mRNA levels in the lung tissues and the protein levels in BAL fluid. Then, a group of mice was intranasally treated either with recombinant murine IL-10 or with vehicle and exposed to cigarette smoke, before the same analysis was performed.

Results: Cigarette smoke exposure provoked pulmonary inflammation in mice, as neutrophil and macrophage counts in BAL fluid were increased (p<0.01). In parallel, the levels of various proinflammatory cytokines including KC and MIP-2 significantly increased (p<0.01), as well as IL-10 expression. The enhancement in the neutrophil counts in BAL fluid caused by smoke exposure was significantly attenuated by the intranasal administration of IL-10 (p<0.05), while that in macrophage counts was not altered.

Conclusion: Our results suggested that IL-10 potentiates the suppression of the neutrophil-associated inflammatory reactions caused by cigarette smoke exposure.

P420

Activation of the inflammasome pathway during exacerbations of COPD

Patricia Sobradillo¹, Rosa Faner², Nestor Soler⁴, Eugeni Ballester⁴, Juan Ignacio Aróstegui³, Jordi Yagüe³, Juan Manel³, Alvar Agustí⁴. ¹CIBERES, Cíberes, Barcelona, Spain; ²Fundación Caubet Cíberes, Fundación Caubet Cíberes, Barcelona, Spain; ³Immunology, Hospital Clinic, Barcelona, Spain; ⁴Thorax Institute, Hospital Clinic, Barcelona, Spain

Introduction: Exacerbations of COPD (ECOPD) are characterized by a burst of inflammation. The inflammasome is an intracellular sensing mechanism that leads to activation of caspase-1 and processing of pro IL-1β and pro IL-18 into their mature forms.

Aim: To investigate if the inflammasome pathway is activated in ECOPD patients. **Methods:** We studied 10 COPD patients hospitalized because of ECOPD, 7 of whom were reevaluated 3 months after discharge (sECOPD), 13 patients with clinically stable COPD (SCOPD), 8 smokers with normal lung function (S) and 11 non smokers (NS). We determined: (1) in serum: caspase-1, IL-1β, IL-18, IL-1Ra, IL-6, IL-8 and TNF-α; (2) in circulating monocytes and lymphocytes: intracellular activity of caspase-1 (basally and after stimulation with inflammasome ligands: ATP, Nigericine and Cigarette Smoke Medium); and (3) in whole blood: caspase-1 and NLRP3 mRNA.

Abstract P420 – Table 1

		ECOPD (n=10)	sECOPD (n=7)	SCOPD (n=13)	S (n=8)	NS (n=11)
Serum	Caspase-1, pg/ml	175.0±77.6 [§]	127.2±53.3	124.3±80.3	156.6±79.6 [#]	89.40±29.4
	IL-1Ra, pg/ml	901.2±594.0 [§]	729.2±280.9	583.9±404.3*	819.1±517.5 [#]	382.3±74.2
	IL-18, pg/ml	426.4±157.2 [§]	332.8±154.1	329.8±100.9	295.6±88.1	275.1±67.1
Monocytes	Caspase-1 Basal activity MFI	122.5±36.2 [§]	173.7±59.4	144.0±33.0 [#]	156.4±15 [#]	187.0±43.04
Blood mRNA	Caspase-1, RQ	0.077±0.08	0.05±0.05	0.09±0.15	0.04±0.04	0.077±0.08
	NLRP3, RQ	0.27±0.38	0.04±0.04	0.22±0.37	0.16±0.31	0.2±0.2

MFI = mean fluorescence intensity, RQ = relative quantification. Symbols indicate p<0.05 (ANOVA): NS vs. S[§]; S vs ECOPD[‡]; NS vs. ECOPD[§]; SCOPD vs. ECOPD^{*}; NS vs. SCOPD[#]; ECOPD vs. sECOPD.

Results: Table 1 shows main results (mean ± SD).

Conclusions: The inflammasome pathway participates in the inflammatory burst that characterizes ECOPD.

P421

Effect of budesonide on fibroblast-mediated collagen gel contraction and degradation

Qihong Fang¹, Nancy Schulte², Xingqi Wang¹, Anna Miller-Larsson³, Elisabet Wieslander³, Xiangde Liu¹, Myron Toews², Stephen Rennard¹. ¹Pulmonary, Critical Care, Sleep and Allergy Division, University of Nebraska Medical Center, Omaha, United States; ²Pharmacology and Neuroscience, University of Nebraska Medical Center, Omaha, United States; ³R&D, AstraZeneca, Lund, Sweden

Background: The balance between production and degradation of extracellular matrix is crucial in maintaining normal tissue structure. Budesonide is known to have anti-inflammatory effects, but its effect on fibroblast-mediated tissue repair and remodeling has not been fully studied.

Methods: Using human fetal lung fibroblasts (HFL-1 cells) in a three-dimensional collagen gel culture system, the current study investigated the effect of budesonide (1-1000 nM) on collagen gel contraction and degradation in the presence or absence of inflammatory cytokines (IL-1β and TNF-α; 5 ng/ml each) and, in order to activate latent proteases, the serine protease trypsin (0.25 μg/ml).

Results: Inflammatory cytokines significantly inhibited collagen gel contraction mediated by lung fibroblasts. Budesonide counteracted the effect of cytokines in a concentration-dependent manner (to 50%, p<0.01). Budesonide (100 nM) almost completely inhibited the release and mRNA expression of metalloproteinases (MMP) -1, -3 and -9 induced by the cytokines (p<0.05). Exposure to the cytokines plus trypsin increased collagen degradation and activation of the MMPs. Budesonide blocked both the enhanced collagen degradation (p<0.01) and suppressed the trypsin-mediated activation of cytokine-induced MMP-9 and MMP-3. Similar effects were observed with dexamethasone (1 μM), suggesting a class effect.

Conclusions: These findings suggest that budesonide directly modulates contraction of collagen and can decrease collagen degradation under inflammatory conditions through suppressing release and activation of MMPs. By modulating the release and activity of MMPs, inhaled budesonide may be able to modify airway tissue repair and remodeling.

P422

Serum lipid profile and iron content in serum and pulmonary tissue in experimental pulmonary emphysema

Snezana Zunic-Bozinovski¹, Svetlana Zunic², Slavko Vucicevic³, Ivan Milovanovic¹, Danica Djarmati⁴, Gordana Djordjevic-Denic¹. ¹Institute for Pathophysiology, School of Medicine, Belgrade, Serbia; ²Institute for Nuclear Medicine, Oncology Unit, Clinical Center of Serbia, Belgrade, Serbia; ³Radiotherapy Unit, Institute of Oncology and Radiology of Serbia, Belgrade, Serbia; ⁴Trace Elements Unit, Health Protection Institute of Serbia, Belgrade, Serbia

Disturbed lipid metabolism and some trace elements, including iron, may be involved in pathogenesis of experimental pulmonary emphysema via different mechanisms. We studied serum lipid profile and iron content in serum and pulmonary tissue of rabbits with experimental pulmonary emphysema. The study was done on three groups of animals: E (n=16) – experimental group on hypercholesterolemic diet (4% crystalline cholesterol in edible oil solution), C (n=16) – control group on standard diet for that animal species, and O (n=13) – group on oily diet. Serum lipid profile (total cholesterol, triglycerides, HDL, VLDL and LDL cholesterol fractions) was determined by enzymatic colorimetric method and iron content in serum and pulmonary tissue by atomic absorptive spectrophotometric method. Pulmonary emphysema was pathohistologically confirmed. There was a significant increase in total serum cholesterol (p<0.001 and p<0.05 respectively) and LDL cholesterol (p<0.001) in E compared to both C and O group. Serum iron concentration was increased in E in comparison with C group (p<0.05). In pulmonary tissue iron content was decreased in both E and O group compared to C group (p<0.01 and p<0.001 respectively), as well as in O in comparison with E group (p<0.001). The results of present study indicate that disturbed lipid metabolism and iron content alterations could be in association with oxido-reductive and metabolic disbalance, connected to emphysematous alterations in pulmonary tissue of investigated animals.

P423

Inhibition of β -adrenoceptors alleviated mucus secretion and emphysema in cigarette smoke exposed rat model

Fei Wang¹, Ji-Hong Li², Bei He³. ¹Pulmonary Department, Peking University Third Hospital, Beijing, China; ²Pulmonary Department, Beijing Shijitan Hospital, Beijing, China; ³Pulmonary Department, Peking University Third Hospital, Beijing, China

Background: The sympathetic nervous (SN) is probably over activated in COPD. Some retrospective studies found that treatment with β -blockers may reduce the risk of exacerbations and improve survival in COPD patients. However, our preliminary data showed that no significant change of lung function in COPD patients after treatment with β -blocker. The aim of the study is to elucidate whether inhibition of the SN over activation may improve lung pathological change in smoke exposed rat model.

Methods: The rats were randomly divided into 3 groups: control group (C, n=9), smoke-exposed group (S, n=8) and smoke-exposed plus propranolol group (S/P, n=8). S and S/P rats were exposed to cigarette smoke for 3 months, and S/P rats were further treated with propranolol for 1 month. Lung tissue pathologic score and mean linear intercept (MLI) were determined.

Results: The scores of mucus secretion, goblet-cell metaplasia, smooth muscle cells (SMC) proliferation, and MLI were significant different in the 3 groups (see table 1), however, no significant difference was found in other aspects of pathological changes.

Table 1. Lung tissue pathologic scores in the 3 groups (mean \pm SD)

Group	Mucus secretion	Goblet-cell metaplasia	SMC proliferation	MLI (μ m)
C	7.68 \pm 5.03	0.13 \pm 0.04	0.23 \pm 0.18 [†]	30.33 \pm 2.20 [‡]
S	17.64 \pm 4.62 [§]	1.66 \pm 0.79 [¶]	0.79 \pm 0.55 [§]	47.46 \pm 4.98 [¶]
S/P	9.78 \pm 5.98 [*]	0.96 \pm 0.55	0.83 \pm 0.71	39.47 \pm 2.56 [†]
P value	0.006	0.000	0.021	0.000

*P<0.05 for S/P vs S, [†]P<0.001 for S/P vs S, [‡]P<0.01 for S vs C, [§]P<0.001 for S vs C, [¶]P<0.05 for C vs S/P, ^{††}P<0.001 for C vs S/P.

Conclusion: In cigarette smoke exposed rat model, inhibition of β -adrenoceptors alleviated mucus secretion and emphysema.

P424

AMPK signalling regulates Nrf2 localization and activity via sirtuins in a monocytic cell line

Laura Nunez Naveira, Nicolas Mercado, Kazuhiro Ito. *Airway Disease, National Heart and Lung Institute (NHLI), Imperial College, London, United Kingdom*

Nrf2 participate in the defence against oxidative stress by inducing anti-oxidant genes such as HO-1 and NQO1, which system is downregulated in COPD. AMPK and sirtuins are ageing related signals reported to control the expression of genes. Sirt1 is also down-regulated in COPD lung.

Aims: To evaluate the effect of sirtuin inhibition and AMPK activation/inhibition on the nuclear localization and activity of Nrf2, and the expression of HO1 and NQO1.

Methods: THP-1 monocytic cells were treated with a sirtuin inhibitor (sirtinol), hydrogen peroxide (H2O2) as oxidative stress reagent and AICAR and dorsomorphin as AMPK activator and inhibitor respectively. Nrf2 protein levels were measured by western blot and the samples were normalized against Lamin A/C. The mRNA levels of HO1 and NQO1 were measured by RT-PCR and normalized against GNB2L1. Nrf2 binding capacity was measured by ELISA.

Results: H2O2 increased Nrf2 in the nucleus by 2-fold and pretreatment with sirtinol did not affect this induction. In contrast, although Nrf2 binding activity also increased by 50% with H2O2, preincubation with sirtinol abrogated this activation. Dorsomorphin treatment provoked a decrease in the nuclear levels of Nrf2 by 30%. In contrast, AICAR increased Nrf2 in the nucleus by 30% and augmented the mRNA levels of HO1 and NQO1 by 3.3 and 1.6-fold respectively.

Discussion: Sirtuins depletion observed in COPD may cause a reduction of the anti-oxidative stress capacity by affecting Nrf2 regulation of HO1 and NQO1. AMPK may be participating in this oxidative stress defence. These results highlight the importance of the sirtuins/AMPK axis in COPD development and its possible use as targets for therapeutic purposes.

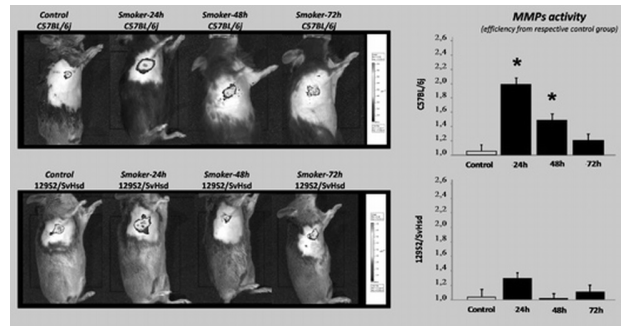
P425

Molecular markers involved in susceptibility to lung injury after acute exposure to tobacco smoke in different mouse strains. Fluorescent molecular in vivo imaging application

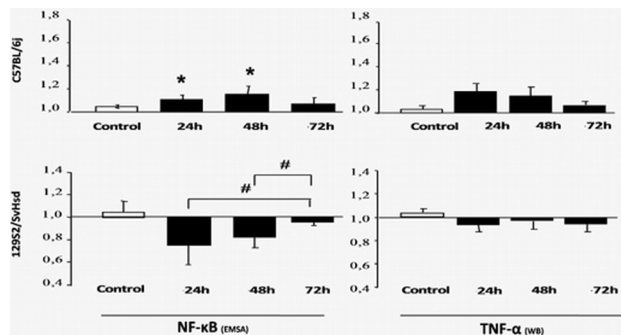
Sandra Pérez-Rial, Laura del Puerto-Nevado, Álvaro Girón-Martínez, Raúl Terrón-Expósito, Nicolás González-Mangado, Germán Peces-Barba. *Pulmonology, IIS-Fundación Jiménez Díaz, Madrid, Spain*

Mouse strains C57 and 129S2 show different susceptibility to developing COPD when they are exposed chronically to tobacco smoke. The aim of this study is to determine molecular differences, between these two strains in the early stages of the disease within 24, 48 and 72h of being acutely exposed to tobacco. We quantify the levels of MMP-12 and analyze MMPs activity in lungs of mice by fluorescence molecular imaging techniques using MMPs activable fluorescent probe. We also

measure NF- κ B and TNF- α levels. The results in C57 strain showed a significant increase in MMPs activity measured at 24 and 48h of cigarettes exposure while 129S2 did not observed changes.



In addition, MMP-12 levels increased significantly in C57, but not in 129S2 strain. Respect to the studied of NF- κ B, significantly increased at 24 and 48h, in correlation with an increase in the levels of TNF- α , only in C57.



These results may explain the increased tobacco smoke susceptibility in early stages to develop lung emphysema with the C57 strain, as compared to the less susceptible strain, 129S2.

P426

Effects of a protease inhibitor from the tick *rhhipicephalus boophilus microplus* in an experimental model of emphysema

Clarice Olivo¹, Luana Neves¹, Juliana Lourenço¹, Francine Almeida¹, Carla Prado², Iolanda Tibério¹, Aparecida Tanaka³, Sergio Sasaki⁴, Milton Martins¹, Fernanda Lopes¹. ¹Department of Medicine, Universidade de São Paulo, Sao Paulo, Brazil; ²Department of Biological Science, Universidade Federal de São Paulo, Sao Paulo, Brazil; ³Department of Biochemistry, Universidade Federal de São Paulo, Sao Paulo, Brazil; ⁴Human and Natural Science, Universidade Federal do ABC, Sao Paulo, Brazil

Although COPD is one of the leading causes of morbidity and mortality in the world, until now there is no effective treatment to improve lung function in COPD patients. Animal models have been employed to better understand the pathophysiology of this disease and to test new therapies.

Objectives: To evaluate the effects of a protease inhibitor in emphysema.

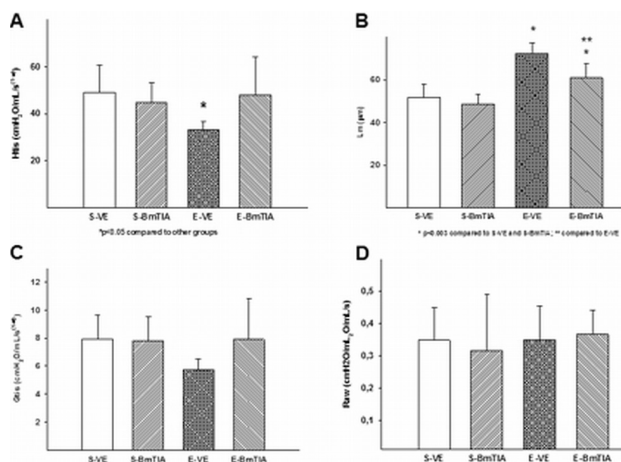


Figure 1

SUNDAY, SEPTEMBER 25TH 2011

Methods: 40 C57BL/6 mice received either a nasal instillation of 50 μ l (0.667 UI) of porcine pancreatic elastase (PPE) or saline (S) and 1h after, animals received a second nasal instillation of 50 μ l of either a protease inhibitor (r-BmTIA, 35.54pmol) or vehicle (VE). After 21 days, animals were anesthetized and airway resistance (RAW), tissue damping (Gtis) and tissue elastance (Htis) were measured using Flexivent small animal ventilator. After sacrifice, lungs were removed and formalin-fixed for posterior analysis of mean linear intercept (Lm).

Results: We observed a decrease in Htis values only in PPE-VE group [Fig. 1A]. Lm values were increased in PPE-VE and PPE-BmTIA groups, whereas the PPE-BmTIA showed a decrease in Lm values compared to PPE-VE group [Fig. 1B]. There was no difference in Gtis and Raw values [Fig. 1C and Fig. 1D, respectively].

Conclusions: We showed that a single dose of this protease inhibitor attenuated parenchyma destruction in an experimental model of emphysema. Supported by FAPESP, LIMHC-FMUSP, CNPq, Brazil.

P427

HDAC2-independent anti-inflammatory effects of budesonide in human lung fibroblasts

Xingqi Wang¹, Amy Nelson¹, Anna Miller-Larsson², Elisabet Wieslander², Xiangde Liu¹, Stephen Rennard¹. ¹*Pulmonary, Critical Care, Sleep and Allergy Division, University of Nebraska Medical Center, Omaha, United States;* ²*R&D, AstraZeneca, Lund, Sweden*

Background: Reduced response of COPD patients to the anti-inflammatory therapy with glucocorticoids may be due to reduced expression of histone deacetylase (HDAC) in alveolar macrophages and bronchial epithelial cells as suggested by recent studies. Lung fibroblasts release inflammatory mediators and are key cells in tissue remodeling following airway inflammation. However, HDAC expression and its role in mediating glucocorticoid effect on fibroblast functions have not been studied.

Methods and results: Human fetal lung fibroblasts (HFL-1) were exposed to IL-1 β +TNF- α (1ng/ml each), which stimulated release of IL-6, IL-8 and metalloproteinases MMP-1 and MMP-3. These responses were inhibited by the glucocorticoid budesonide (0.1-100nM) in a concentration-dependent manner. An HDAC inhibitor (trichostatin A) did not reverse the effects of budesonide on release of cytokines and MMPs in HFL-1 cells while it blocked the inhibitory effects of budesonide in human bronchial epithelial cells and monocytes. Furthermore, siRNA targeting HDAC2 did not interfere with the inhibitory effects of budesonide on HFL-1 MMP release. Exposure to cigarette smoke extract (5%) did not affect HDAC2 protein expression in HFL-1 cells and did not interfere with the budesonide effects. Finally, there was no statistically significant difference between COPD and control subjects in HDAC2 expression and the effects of budesonide on cytokine or MMP release from lung fibroblasts.

Conclusions: HDAC2 is not required for budesonide to inhibit MMP and cytokine release by lung fibroblasts. These results also suggest that budesonide has a potential to counteract fibroblast-mediated tissue remodeling following airway inflammation in COPD.

P428

The nuclear liver X receptor and its role in smoke exposed rat lungs

Adelheid Kratzer, Jonas Salys, Martin Zamora, Laima Taraseviciene-Stewart. *Medicine, Pulmonary Sciences and Critical Care Medicine, Denver, CO, United States*

Background: Chronic obstructive pulmonary disease (COPD) is one of the leading causes of mortality worldwide and currently there are no efficient treatments. Liver X receptor (LXR) plays a role not in lipid metabolism and inflammation.

Objectives: To investigate the effects of CS on LXR activation and target gene regulation in vivo and in vitro and to study potential protective effects of LXR activation against CS-induced emphysema.

Methods: Sprague Dawley rats were exposed to second hand CS for two months. The lung tissue and bronchoalveolar lavage (BAL) cells were examined for LXR target gene expression and emphysema development was measured by MLI. Rat AM and EC treated with CS extract, LPS or DMHCA, a steroidal LXR agonist were examined for LXR target gene and protein expression and migratory capacity. LXR localization before and after CS exposure was determined by immunofluorescence. **Results:** Whereas expression of ATP binding cassette transporter A1, a known LXR target gene, was not changed in the lung, but significantly downregulated in the BAL cells from CS-exposed rat lungs and in CS extract-treated AM. Immunofluorescence showed reduced nuclear localization of the anti-inflammatory LXR β isoform in AM and pulmonary EC. Activation of LXR attenuated LPS-induced genes such as COX2, CCL5 and IL18, but upregulated IL18 binding protein (IL-18BP), an endogenous IL-18 inhibitor. It increased the migratory capacities of rat alveolar macrophages towards CCL5.

Conclusions: Activation of LXR attenuated the pro-inflammatory cytokine production by AM suggesting that LXR agonist treatment might be beneficial to prevent and treat CS-emphysema.

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P429

Insulin-dependent PI3-Kinase/Akt and ERK signaling pathways inhibit TLR3-mediated HBEC apoptosis

Takanori Numata¹, Jun Araya¹, Satoko Fujii¹, Hiromichi Hara¹, Naoki Takasaka¹, Yoko Yumino¹, Makoto Kawaishi¹, Jun Hirano², Makoto Odaka², Toshiaki Morikawa², Katsutoshi Nakayama¹, Kazuyoshi Kuwano¹. ¹*Department of Internal Medicine, Division of Respiratory Diseases, Jikei University School of Medicine, Tokyo, Japan;* ²*Division of Chest Surgery, Jikei University School of Medicine, Tokyo, Japan*

Introduction: TLR 3 mainly recognizes viral-associated dsRNA. However, recognition of dsRNA byproducts released from apoptotic and necrotic cells is a recently proposed mechanism for the amplification of virulence, suggesting a pivotal participation of TLR3 not only in viral infection, but also in lung diseases where apoptosis plays a critical role, such as asthma and COPD. In addition to metabolic control, insulin signaling has also been postulated to be protective by inhibiting apoptosis.

Aims: We explored the role of insulin signaling in protecting human bronchial epithelial cells (HBEC) against TLR3-mediated apoptosis.

Methods: For the experiments of apoptosis induction by polyinosinic-polycytidylic acid (poly (I:C)), a dsRNA analog, HBEC and airway fragments were treated in the presence or absence of insulin. Knock down of TLR3 was performed by siRNA transfection. Wortmannin (PI3-kinase inhibitor) and PD98059 (MEK inhibitor) were used to elucidate the role of insulin-dependent signaling pathways.

Results: Significant TLR3-mediated apoptosis was induced by poly (I:C), via caspase-8-dependent mechanisms. However, insulin efficiently inhibited TLR3/poly (I:C) induced HBEC apoptosis via PI3-kinase/Akt and ERK pathways, at least partly via the up-regulation of cellular FLICE-inhibitory proteins (cFLIPs) and additionally through protein synthesis-independent mechanisms.

Conclusions: These results implicate TLR3-mediated dsRNA-induced apoptosis in apoptosis-driven lung disease pathogenesis and provide evidence for a novel protective role of insulin.

P430

Overexpression of RAGE in lungs of patients with COPD: A contributor to oxidative stress in COPD

Lei Chen, Lin Liu, Tao Wang, Fuqiang Wen. *Department of Respiratory Medicine, West China Hospital, Chengdu, China*

Background: Receptor for advanced glycation end products (RAGE) has been recently suggested to be implicated in COPD, although the mechanisms remain unclear. The aim of this study is to investigate the expression of RAGE in lung tissues from COPD patients and its potential role in oxidative stress in COPD.

Methods: Peripheral lung tissue specimens were obtained from 40 patients who underwent lung resection for non-small cell lung cancer, including 8 non-smoker controls, 7 non-COPD smokers and 25 smoker COPD patients. Immunohistochemistry and ELISA were used to assess RAGE expression in lung tissues, and oxidative stress was also measured by ROS and GSH. Furthermore, human pulmonary epithelial cell (A549) and bronchial epithelial cell (16HBE) were cultured with cigarette smoke extract (CSE). Neutralizing antibody against RAGE was used to detect the role of RAGE in oxidative stress in COPD.

Results: RAGE expression level in smoker COPD patients was significantly higher than non-smoker controls and non-COPD smokers, which was dominantly expressed in the bronchiolar and alveolar epithelia. Importantly, RAGE in smoker COPD patients was positively correlated with oxidative stress, evidenced by levels of ROS and GSH. In the in-vitro study, CSE enhanced oxidative stress level in A549 and 16HBE cells, as well as activation of NF- κ B, a key redox transcription factor, which were all reversed by pretreatment of anti-RAGE antibody.

Conclusion: Overexpression of RAGE may contribute to COPD pathogenesis, at least partly through enhancement of oxidative stress level.

P431

MMP-mediated regulation of EnaC channel in pleural mesothelium

Eleni Apostolidou¹, Foteini Malli², Efosini Paraskeva¹, Konstantinos Gourgouliani², Paschalis-Adam Molyvdas¹, Chrissi Hatzoglou¹. ¹*Physiology Department, University of Thessaly, Larissa, Greece;* ²*Respiratory Department, University Hospital of Thessaly, Larissa, Greece*

Introduction-aim: The epithelial sodium channel (ENaC), which participates in sodium reabsorption across the apical membrane of mesothelial cells, has been recently identified in pleura with molecular techniques. MMP2 and MMP9 are two matrix metalloproteinases (MMPs) that have been found elevated in exudative pleural effusions. The aim of this study was to investigate if MMP2 and MMP9 influence functionally ENaC activity.

Methods: Intact sheets of parietal sheep pleura were obtained and the short-circuit current (Isc) was recorded in Ussing chambers. MMP2 (0.1 ng/ml or 20 ng/ml) or MMP9 (0.1 ng/ml or 20 ng/ml) were added on the apical solution of the pleura. After a 40-min incubation, amiloride (10⁻⁴ M) was added to the apical compartment of all experiments in order to calculate the ENaC-mediated current. This current was defined as the difference between the Isc value just before amiloride addition and the Isc value 5 min after amiloride addition. The amiloride-sensitive Isc was compared between control tissues (having received only PBS) and tissues treated with MMP2 or MMP9.

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Results: A statistically significant decrease in amiloride-sensitive I_{sc} occurred when 20 ng/ml MMP2 were added to the pleura ($p < 0.05$). When the pleura was incubated with 0.1 ng/ml MMP2 or MMP9, an increase in amiloride-sensitive I_{sc} occurred but this was not statistically significant.

Conclusions: According to the above results, MMP2 regulates ENaC activity by decreasing the sodium current which is produced by ENaC. A same effect for MMP9 was not confirmed. Previous studies have shown a serine-peptidase mediated regulation of ENaC activity. This is the first study that suggests a matrix metalloproteinase-mediated downregulation of ENaC current.