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USE OF DUAL RNA-SEQ IN VIVO AS A SCREENING APPROACH TO IDENTIFY NEW TARGETS FOR NEW ANTIMICROBIAL DRUGS

B. Euba[1,2], N. López-López[1], L. Caballero[1], C. Gil-Campillo[1], R. Díez-Martínez[3], M. Barbier[4] and J. Garmendia[1,2]

[1]Instituto de Agrobiotecnología (IdAB), CSIC-Gobierno de Navarra, Spain. [2]CIBER de Enfermedades Respiratorias (CIBERES), Madrid, Spain. [3]Ikan Biotech S.L., The Zebrafish Lab., Centro Europeo de Empresas e Innovación de Navarra (CEIN), Spain. [4]Department of Microbiology, Immunology and Cell Biology, West Virginia University School of Medicine, Morgantown, USA.

Introduction: Respiratory diseases represent a major threat to public health. Of the 56.9 million deaths worldwide in 2016, 54% were due to the top 10 leading causes, being chronic obstructive pulmonary disease (COPD) and lower respiratory tract infections (LRTI) in top positions 3 and 4, respectively. The Gram negative bacterium nontypeable Haemophilus influenzae (NTHi) is typically a benign commensal of the human nasopharynx, but also an opportunistic pathogen. NTHi is a major causative agent of LRTI, the most common bacterial cause of infection of the lower airways in adults with COPD, and it is responsible for approximately half of bacterial exacerbations, which, altogether requires novel and more efficient treatments. Profiling gene expression changes that accompany infection is key to understand pathogenesis, and a tool for screening of new targets for new drugs.
Objectives: To understand the NTHi host-pathogen interplay by in vivo dual RNA-seq.
Methods: NTHi strain 375 was grown in BHI medium supplemented with hemin and β-NAD (sBHI) (Sample 1), and used to intranasally infect CD1 mice, which were sacrificed at 12 hpi. Lung homogenates (Sample 2) and filtered bronchoalveolar lavage (BALF) samples (Sample 3) were processed. Non-infected lung homogeneates (Sample 4) were also processed. RNA was extracted with NucleoSpin RNA kit; RNA integrity was verified with a Bioanalyzer. The kit Ribozero epidemiology was used for depletion of eukaryotic and prokaryotic rRNA. Libraries were prepared with ScriptSeq gold epidemiology. Three independent biological samples were used to generate three libraries per condition. Samples were sequenced on a Hiseq platform with 2×150bp reads. RNA reads were trimmed and mapped in pairs to H. influenzae and Mus musculus genomes independently using the software CLC Genomics (Qiagen). The number of reads obtained from each group, and the percentage of the reads mapped to the pathogen or to the murine host, indicated that the methodology used provided RNA of sufficient quality and quantity to allow transcriptomic analysis of both pathogen and host during respiratory infection in vivo. RPKM were calculated to determine relative changes in gene expression (*P*-value of 0.05). GO term, DGE, STRING, and KEGG analyses were performed to identify sets of genes differentially expressed between conditions.
Conclusions: Data analysis by comparing Sample types 1 to 3 shows overexpression of oligoelement uptake system (Fe, Zn, Mo)-encoding genes. Also, 11 out of 14 genes encoding the purine de novo biosynthesis pathway, and 9 out of 26 natural competence encoding genes are overexpressed upon infection, in a similar manner to that observed when comparing NTHi transcriptome when grown in sBHI and MIV media.[1] MIV is the reference medium used to induce NTHi natural competence in laboratory conditions, which suggests that the host airways may provide suitable conditions for DNA uptake by NTHi. Sample 2 could not be used for bacterial profiling due low % mapping with the H. influenzae genome, and will be used for further host gene expression profiling.

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ARE THERE DIFFERENCES IN THE INNATE RESPONSE BETWEEN BRONCHIOLITIS AND PEDIATRIC RECURRENT WHEEZE?

B. Sastre[1,2], J.M. Rodrigo-Muñoz[1,2], I. Mora[1], J.A. Cañas[1,2], D.A. García-Sánchez[1], M.L. García-García[3,4,5], C. Calvo[4,5,6,7] and V. del Pozo[1,2]

[1]Department of Immunology, IIS-Fundación Jiménez Díaz, Madrid, Spain. [2]CIBER de Enfermedades Respiratorias (CIBERES), Madrid, Spain. [3]Pediatrics Department, Severo Ochoa Hospital, Leganés, Spain. [4]Translational Research Network in Pediatric Infectious Diseases (RITIP). [5]Alfonso X El Sabio University, Madrid, Spain. [6]Pediatric Infectious Diseases Department, Hospital Universitario La Paz, Madrid, Spain. Fundación IdiPaz, Madrid, Spain. [7]TEDDY Network (European Network of Excellence for Pediatric Clinical Research).

Introduction: Infants affected by bronchiolitis during first months of life, frequently develop recurrent wheezing and asthma. The aim of this study was to analyse the innate immune response that characterize bronchiolitis (BQ) and recurrent wheezing (RW). Several hypotheses have been suggested such as the existence of a defective Th1 immune response in this first viral respiratory infection or the possibility that Th2 immune response take precedence, exacerbat-

ing this response later agents or antigens. However, a clear photograph of immune environment in both situations is yet not clear.
Objectives: The aim of this study was to analyse the innate immune response that characterize bronchiolitis (BQ) and recurrent wheezing (RW).
Methods: Ninety-nine and seventy-two infants hospitalized with diagnosis of BQ or RW respectively between October 2016 and August 2017, and 23 healthy infants were included. Nasopharyngeal aspirates (NPA) were used as main sample employed, dividing them in two phases: supernatant and cellular pellet. Type 2 innate lymphoid cells (ILC2) were evaluated by flow cytometry. Twenty-eight pro-inflammatory factors linked to innate immunity, inflammation and epithelial damage were evaluated in NPA supernatant by classical ELISA and Luminex. Also, eleven genes were analyzed in NPA cells by quantitative PCR (qPCR).
Results: Bronchiolitis presented a significant increase of ILC2 percentage compared with RW ($P < 0.05$). ST2-positive ILC2 percentage was higher in BQ vs. RW group ($P < 0.01$). TLR3, IL-33, IFNγ, IL-10, and FLG (filaggrin) gene expression was significantly increased in BQ in relation to RW group (TLR3, IL-33: $P < 0.001$; IFNγ, IL-10, FLG: $P < 0.05$). In supernatant, we observed a significant increase of IFNγ in BQ compared to RW group ($P < 0.05$), augmented levels of IL-10 in both pathologies compared to healthy population ($P < 0.001$). TSLP in BQ showed a higher level than other groups. Elevated levels of periostin in BQ and RW groups compared to control were observed, reaching statistical significance between RW and healthy populations. Rest of molecules not showed statistically significant differences.
Conclusions: Differential innate immune response and epithelial repair mechanisms were observed between both diseases; however, BQ and RW share mechanisms such us monocyte activation, vascular damage and fibroblast repair.

EFFECT OF PHOSPHODIESTERASE 4B INHIBITOR GSK256066 ON LEUKOCYTE/ENDOTHELIUM INTERACTIONS FROM CHRONIC OBSTRUCTIVE PULMONARY DISEASE: AN IN VITRO STUDY

I. Roger[1], A. Morell[2], P. Ribera[2], R. Guijarro[3] and J. Cortijo[1,2]

[1]CIBER de Enfermedades Respiratorias (CIBERES), Madrid, Spain. [2]Pharmacology Department, Valencia University, Valencia, Spain. [3]Hospital General de Valencia, Valencia, Spain.

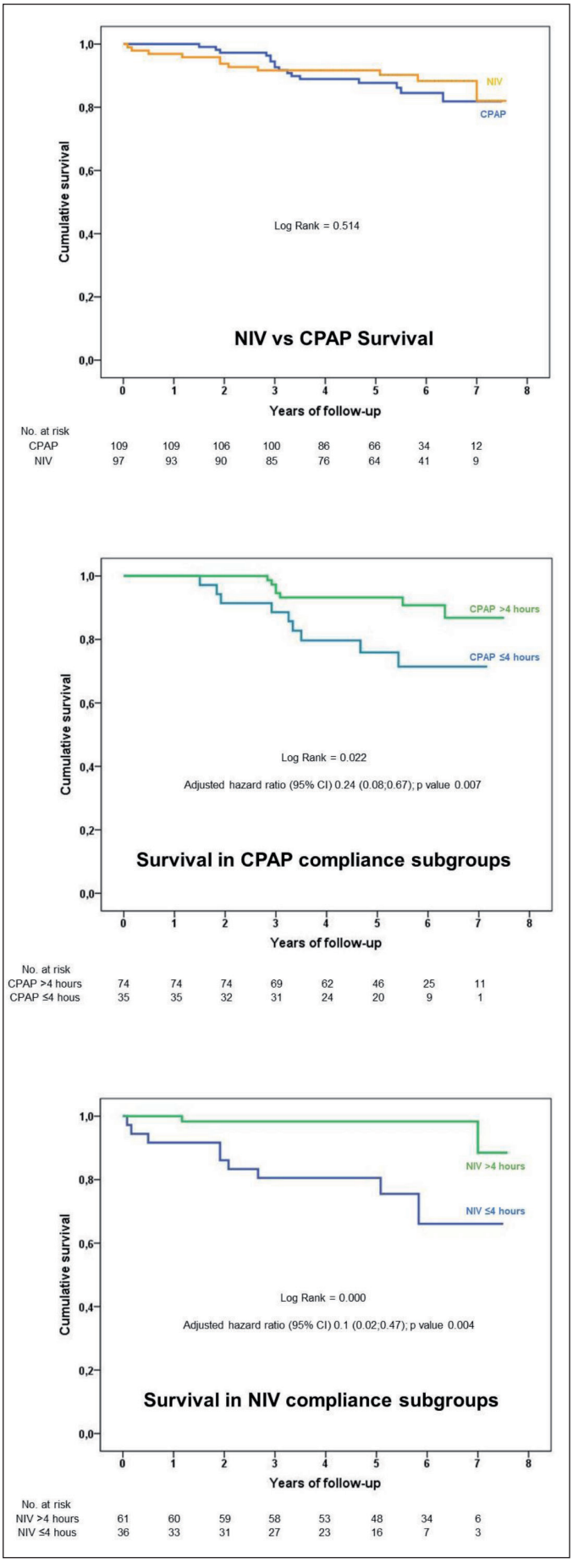
Introduction: Chronic obstructive pulmonary disease (COPD) is a progressive inflammatory lung disease characterized by an accumulation of circulating leukocytes in lung tissue. The phsosphodiestare 4 (PDE4) inhibitor roflumilast has been approved for the treatment of severe COPD at risk of exacerbations as an add on therapy to bronchodilators and inhaled corticosteroids. However, side effects including gastrointestinal limit the clinical use. Therefore there is a need to study more selective PDE4 drugs targeting different PDE4 isoforms, such as PDE4B to improve efficacy without side effects.
Objectives: The aim of this study was to demonstrate the efficacy of GSK256066, a selective PDE4B inhibitor on leukocyte-endothelial cell interactions in vitro.
Methods: Human pulmonary artery endothelial cells were grown in monolayers and pre-incubated with rofllumilast, dexamethasone or GSK256066 at different concentrations (0.1 nM-10 µM). After 30 minutes, cells were stimulated with cigarette smoke extract 5% (CSE) during 24 hours. Monocytes, neutrophils and whole blood from healthy subjects and COPD patients were used to study leukocyte/endothelial adhesion by flow chamber. Adhesion molecules and cytokine release were measured by RT-PCR and ELISA, respectively. P-p38 and p-ERK1/2 was measured by Western-Blot.
Conclusions: GSK256066 inhibited monocyte/endothelial cell interactions in leukocytes from healthy and COPD with IC50 values of 2.18 ± 0.1 and 3.16 ± 0.2 respectively that were higher than that observed with roflumilast (61.5 ± 0.2 and 7.94 ± 0.3) and dexamethasone (49.7 ± 0.2 and 398.11 ± 0.9). Results from whole blood leukocyte and neutrophil/endothelial interaction were similar to those observed for monocyte/ leukocyte interaction indicating higher potency for GSK256066 compound. CSE increased IMCAM-1, VCAM-1 and E-selectin in endothelial cells that were inhibited by GSK256066 and in a lesser extent by roflumilast and dexamethasone. GSK256066 was more potent than roflumilast and dexamethasone inihibiting IL-8, GM-CSF and MCP1 release, and phosphorylation of p38 and erk1/2. The selective PDE4B inhibitor GSK256066 shows more potent inhibitory effects than other anti-inflammatory therapies used in COPD such as roflumilast and dexamethasone on leukocyte/ endothelial interactions, which may represent a promising therapeutic option.

CONTINUOUS POSITIVE AIRWAY PRESSURE VS NONINVASIVE VENTILATION FOR LONG TERM TREATMENT OF OBESITY HYPOVENTILATION SYNDROME: THE RESULTS OF THE PICKWICK RANDOMIZED CONTROLLED TRIAL

M. Orta Caamaño[1], M. Sánchez[2,3,4], B. Mokhlesi[5], J. Corral[1,3,4], M. Alonso[3,5], E. Ordaz[6], M. Troncoso[3,7], M. González[8], S. López-Martín[9], J.M. Marín[3,10], S. Martí[3,11], T. Díaz Cambriles[3,12], E. Chiner[13], F. Aizpuru[14], C. Egea[3,15] and J.F. Masa[1,3,4]

[1]San Pedro de Alcántara Hospital, Cáceres, Spain. [2]Virgen del Puerto Hospital, Plasencia, Cáceres, Spain. [3]CIBER de Enfermedades Respiratorias (CIBERES), Madrid, Spain. [4]Instituto Universitario de Investigación Biosanitaria de Extremadura (INUBE), Spain. [5]University of Chicago, IL, USA. [6]Burgos University Hospital, Burgos, Spain. [7]IIS-Fundación Jimenez Díaz, Madrid, Spain. [8]Marqués de Valdecilla Hospital, Santander. Spain. [9]Gregorio Marañón Hospital, Madrid, Spain. [10]Miguel Sevet Hospital, Zaragoza, Spain. [11]Vall d'Hebron Hospital, Barcelona, Spain. [12]12 de Octubre Hospital, Madrid, Spain. [13]San Juan Hospital, Alicante, Spain. [14]Unidad de Investigación de Osakidetza, Álava, Spain. [15]Álava University Hospital IRB, Vitoria, Spain.

Introduction: Noninvasive ventilation (NIV) and continuous positive airway pressure (CPAP) are the most commonly prescribed treatment modalities for patients with obesity hypoventilation syndrome (OHS). Despite differences in cost and complexity between NIV and CPAP, there are no long-term effectiveness studies comparing the two treatment modalities.
Objectives and methods: We performed a large multicenter randomized open-label controlled trial (Pickwick study) to determine the comparative long-term effectiveness of NIV and CPAP using hospitalization days as the primary outcome measure. Sequentially screened OHS patients with severe obstructive sleep apnea (OSA) were randomized to NIV or CPAP and were followed for at least three years. Hospital resource utilization, mortality, incident cardiovascular events, dropouts, compliance, and side effects were quantified. Statistical analysis was performed using intention-to-treat procedure evaluating the incidence density ratio and survival analysis. Adjusted análisis was performed by Poisson, negative binomial and Cox regressions.
Results: In total, 363 patients were screened, 215 were randomized and 202 were available for primary analysis. The median (IQR) follow-up was 5.42 (2.17) years. Hospitalization days per 100 person-years (95%CI) was 158.5 days (148.3-169.3) for CPAP and 156.2 days (155.5-167.5) for NIV (adjusted hazard ratio 0.68 [0.14-3.35]; $P = 0.638$). Other hospital resource utilization including hospitalizations, emergency department visits and ICU days had similar comparative results. Incident cardiovascular events per 100 personyears



(95%CI) was 5.1 (2.95-8.1) for CPAP and 7.46 (4.62-11.4) for NIV (adjusted hazard ratio 1.4 [0.73-2.71]; *P* = 0.315). Mortality rate was 14.7% for CPAP and 11.3% for NIV (adjusted hazard ratio 0.73 [0.33-1.62]; *P* = 0.439) (see Figure). Dropouts, adherence to positive airway pressure (PAP) therapy and secondary effects were similar between the two groups. Subgroups of PAP treatment adherence analysis (first tertile vs second and third tertiles) showed similar results between NIV and CPAP. Intra-group comparisons, however, revealed significantly improved hospital resource utilization and survival in both NIV and CPAP subgroups that were adherent to therapy (see Figure: Cumulative survival for NIV and CPAP arms (first). Cumulative survival between both adherence subgroups (first tertile 1 vs second and third tertiles) in CPAP (second) and NIV (third) arms. Note that the first tertile corresponds with a treatment use ≤ 4 hours/night and second + third tertiles with > 4 hours/night).

Conclusions: NIV and CPAP have similar long-term effectiveness. Given that CPAP therapy is less costly and easier to implement, it should be the preferred treatment modality for patients with OHS and concomitant severe OSA.

PRECISION MEDICINE IN PATIENTS WITH SLEEP APNEA AND ACUTE CORONARY SYNDROME: PREDICTIVE MODEL OF RESPONSE TO TREATMENT

A. Zapater[1], F. Santamaría-Martos[1], I. Benítez[1], L. Pinilla[1], C. Girón[1], A. Castro-Grattoni[1], F. Barbe[1,2] and M. Sánchez de la Torre[1,2]

[1]Translational Research in Respiratory Medicine Group, Hospital Universitari Arnau de Vilanova & Hospital Universitari Santa Maria, IRB Lleida, Lleida, Spain. [2]CIBER de Enfermedades Respiratorias (CIBERES), Madrid, Spain.

Introduction: Obstructive sleep apnea syndrome (OSA) is a frequent and chronic disease with a prevalence of approximately 15-20% in the adult population in our country and is considered an important public health problem. OSA is characterized by the appearance of repeated episodes of obstructive apneas and hypopneas that occur during sleep. The severity of the disease is mainly defined based on the number of apneas and hypopneas per hour of sleep, the apnea-hypopnea index (AHI). OSA is associated with cardiovascular (CV) disease. In this way, OSA is a frequent condition in patients with CV disease, affecting 40-60% of these patients. In patients with OSA, continuous positive pressure therapy (CPAP) reduces blood pressure, and its effects are related to compliance with treatment and baseline blood pressure. The response to the treatment of OSA with CPAP is heterogeneous. There are no tools to predict the response to treatment for the identification of patients who would respond adequately to CPAP for the improvement of CV morbidity associated with OSAS.

Objectives: The general objective of the study is to characterize the epigenetic profile of CV risk in patients with acute coronary syndrome (ACS) and OSA. For this purpose, a main objective is identified, which consists of identifying the epigenetic molecular profile predictive of response to CPAP treatment in patients with OSAS who have suffered an ACS. In addition, the following secondary objectives are identified: *1)* To evaluate the impact of OSAS on the expression profile of risk epigenetic markers CV; *2)* The impact of CPAP treatment will be assessed on: *i)* Profile of epigenetic markers associated with pathology CV; *ii)* Profile of epigenetic markers associated with metabolic disease; *iii)* Profile of epigenetic markers associated with physiological and pathogenic pathways of OSAS and CV disease; *3)* Functional characterization (in silico and in vitro) of the physiopathological mechanisms of ACS and OSA, and response to treatment with CPAP.

Methods: Longitudinal, randomized, and controlled study. This is a ancillary study from the ISAACC study "Influence of sleep apnea-

hypopnea syndrome on the evolution of acute coronary syndrome. Effect of continuous positive pressure (CPAP) intervention. ") (PI10 / 02763). This study has completed its recruitment and the biological samples are available. The expression profile of circulating microRNAs of the OSAS patients who have suffered an ACS will be identified. In addition, markers closely related to cardiovascular risk will be evaluated: TREM-1, renin, angiotensin, aldosterone. Measurements will be performed at baseline and 12 months after effective CPAP treatment.

Conclusions: A tool based on molecular markers with clinical utility will be identified for predicting an adequate response to CPAP treatment in patient with OSA and ACS, to reduce the risk of developing a new CV event.

MOLECULAR CHARACTERIZATION OF ANTIBIOTIC RESISTANCE MECHANISMS IN METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* FROM INTENSIVE CARE UNITS ENDOTRACHEAL TUBES

R. Cabrera[1,2,3], L. Fernández-Barat[1,2,3], A. Motos[1,2,3], R. López-Aladid[1,3], N. Vázquez[1,3], M. Panigada[4], F. Álvarez-Lerma[5], A. Ceccato[1,2,3], Y. López[6], L. Viña[7], G. Li Bassi[1,2,3], L. Muñoz[6], T. Israel[3], P. Castro[8], J.M. Nicolás[8], E. Zavala[9], J. Fernández[10], I. Rovira[11], J. Vila[6], M. Ferrer[1,2,3] and A. Torres[1,2,3]

[1]Cellex Laboratory, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), School of Medicine, University of Barcelona, Spain. [2]CIBER de Enfermedades Respiratorias (CIBERES), Madrid, Spain. [3]Respiratory Intensive Care Unit, Pneumology Department, Hospital Clínic, Barcelona, Spain. [4]Department of Anesthesiology, Intensive Care and Emergency, UOC Rianimazione e Terapia Intensiva, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico Milan, Italy. [5]Critical Care Department, Critical Illness Research Group (GREPAC), Hospital del Mar, Medical Research Institute (IMIM), Hospital del Mar, Barcelona. Spain. [6]Institute of Global Health of Barcelona, Department of Clinical Microbiology, Hospital Clinic, University of Barcelona, Barcelona, Spain. [7]Servicio de Medicina Intensiva, Hospital Universitario Central de Asturias, Oviedo, Spain. [8]Internal Medicine Intensive Care Unit, Hospital Clínic. Barcelona, Spain. [9]Surgical Intensive Care Unit, Hospital Clínic. Barcelona, Spain. [10]Hepatic Intensive Care Unit, Hospital Clínic, Barcelona, Spain. [11]Cardiovascular Intensive Care Unit, Hospital Clínic, Barcelona, Spain.

Introduction: Hospital-acquired and ventilator-associated pneumonia (VAP) are frequent causes of infection among critically ill patients. VAP is one of the most common hospital-acquired bacterial infections in Intensive Care Units (ICU). Unfortunately, many of the nosocomial bacteria that cause VAP are increasingly difficult to treat. Additionally, the evolution and dissemination of multi- and pan-drug resistant strains leave clinicians few treatment options. Since few antibiotic agents have been approved within the last 15 years, and no novel agents specifically targeting VAP have been approved to date, it is anticipated that this problem will worsen. *Staphylococcus aureus* has been recently reported to be the second most frequently isolated microorganism responsible for ICU-acquired pneumonia, of which 29% are methicillin resistant *Staphylococcus aureus* (MRSA). Respiratory infections caused by MRSA are associated with significant morbidity, mortality and cost. The changing epidemiology of MRSA infections, varying resistance to commonly used antibiotics, and involvement of MRSA in community-associated (CA-MRSA) and hospital associated infections are influencing the use and clinical outcomes of currently available anti-infective agents. The increase of antimicrobial resistance due to the ability of microorganisms to transfer resistance genes and the need to know the mechanisms involved in antimicrobial resistance for the design of new antibiotics, justify this study.

Objectives: To define the ST type and detect the molecular mechanisms of resistance involved in MRSA strains from endotracheal tubes of ICU patients with respiratory infection.

Methods: A prospective observational study was carried out in four European tertiary hospitals (Hospital Clinic, Hospital del Mar, Hospital Universitario Central de Asturias, and The Department of Anesthesiology, Intensive Care and Emergency, U.O.C. Rianimazione e Terapia Intensiva, from the Ospedale Maggiore Policlinico, in Milan, Italy). We collected endotracheal tubes (ETTs) from mechanically ventilated patients with microbiological confirmation of MRSA respiratory infection. A total of 20 strains of MRSA isolated from ETT were characterized. MRSA strains were ST typed by sequence analysis of the following seven housekeeping genes: *arc, aro, glp, pta, tpi, yqi, gmk.* We previously confirmed by conventional PCR the presence of genes distributed in 2% agarose gel. Sanger sequences were analyzed by MEGA 5.0 software and the FASTA format was processed by MLST.net database to concatenate these seven genes and to obtain the ST type. The phylogenetic tree was built by Neighbour-Joining methods. We performed antimicrobial susceptibility test, using the Kirby-Bauer method and the ATCC 25923 *(S. aureus)* as a control, to the currently available antimiocrobial agents against *S. aureus:* vancomycin, linezolid, chloramphenicol, gentamicin, rifampicin, ciprofloxacin, clindamycin, erithromycin, trimethoprim, tetracycline, quinupristin-dalfopristin, fusidic acid and tygeciclin. Interpretation of results was carried out according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST). Molecular characterization of each resistance mechanism was screened by PCR and electrophoresis in 2% agarose gels. The purified PCR products were processed for DNA sequencing as aforementioned. We evaluated the most common resistance mechanism in MRSA.

Results: MRSA ST found were: 22 (40%), 217 (15%), 8 (10%), and with a frequency of 5%: 87, 83, 45, 1,535, 954, 403, and 1,221. All MRSA were susceptible to: vancomycin, quinupristin-dalfopristin, tygeciclin, rifampicin, trimethoprim and chloramphenicol. Overall, 11 out of 20 of MRSA were resistant to three or more different antimicrobials agents. MRSA strains were resistant to: ciprofloxacin (95%), erythromycin (80%), gentamicin (35%), clindamycin (35%), tetracycline (25%), linezolid (15%), and fusidic acid (5%). Seventeen out 20 MRSA presented more than one mechanism of resistance. We found the following resistance mechanism: efflux pump tetK (tetracycline), plasmid-mediated aminoglycoside modified enzyme AAC(6')/APH(2") encoded by aac(6´)/aph (2") gene (aminoglycosides). In 64.3%, 14.3% and 21.4% of MRSA the macrolides resistance was mediated by genes ermC and ermA (encoding methylases) or the efflux pump msrA, respectively.

Conclusions: The evidence for MRSA linezolid resistant is a concern due to very high resistance to second-line antibiotics. In addition, the fact that each strain harbors several resistance mechanisms challenges the effectiveness of antibiotic combinations.

DEFINITION OF DISCRIMINATORY BIOMARKERS OF NONALLERGIC AND ALLERGIC ASTHMA AND THEIR SEVERITY AND THE ROLE OF METHYLATION IN THEIR REGULATION

S. Baos[1], M.A. de Pedro[1], L. Cremades[1], D. Calzada[1] and B. Cárdaba[1,2]

[1]IIS-Fundación Jiménez Díaz-UAM, Madrid, Spain. [2]CIBER de Enfermedades Respiratorias (CIBERES), Madrid, Spain.

Introduction: In a previous study[1] we defined specific genes related with asthma and allergic diseases, by studying the gene-expression of 94 genes in a population composed by 4 groups of subjects: healthy control, nonallergic asthmatic, asthmatic allergic and nonasthmatic allergic patients. The analysis of differential gene-expression between control and patients revealed a set of statistically relevant genes

mainly associated with the disease's severity: CHI3L1, IL-8, IL-10, MSR1, PHLDA1, PI3 and SERPINB2.

Objectives: In this project we analyzed if these genes and their proteins could be potential asthma biomarkers to differentiate between nonallergic asthmatic and asthmatic allergic subjects and if methylation takes part in the regulation of the gene-expression —these results have being protected (patent reference: PCT/ES2018/070515).

Methods: Protein quantification was determined by ELISA or Western Blot. Statistical analyses were performed by unpaired t-test, using the Graph-Pad InStat 3 program. The sensibility and specificity of the gene and protein expression of several candidate biomarkers for differentiating the 2 groups (and their severity) was performed by receiver operating characteristic curve (ROC) analysis using the R program. DNA extracted from PBMCs of the patients with asthma (allergic and nonallergic) was treated with sodium bisulfite and amplified by PCR with primers designed to amplify CpG islands near the promotor region of two of the most significant genes. The methylation analysis was done with the Sequenom EpiTYPER approach.

Results: In the ROC curve analysis, single genes showed a good sensitivity and specificity to discriminate some of the phenotypes. However, interesting combinations of two or three protein biomarkers were found to distinguish the asthma disease and its severity between the different phenotypes of this pathology using easy to measure techniques. The methylation analysis showed statistically significant differences between groups in the 2 genes analyzed.

Conclusions: Gene and protein panels formed by single and combinations of biomarkers have been defined in easy to obtain samples and by standardized techniques, that could be useful to characterize phenotypes of asthma, but specially, to differentiate the severity of the asthmatic disease. A possible regulatory mechanism of molecular biomarkers of asthma has been defined, being methylation a possible key factor for the differential gene-expression of asthma patients.

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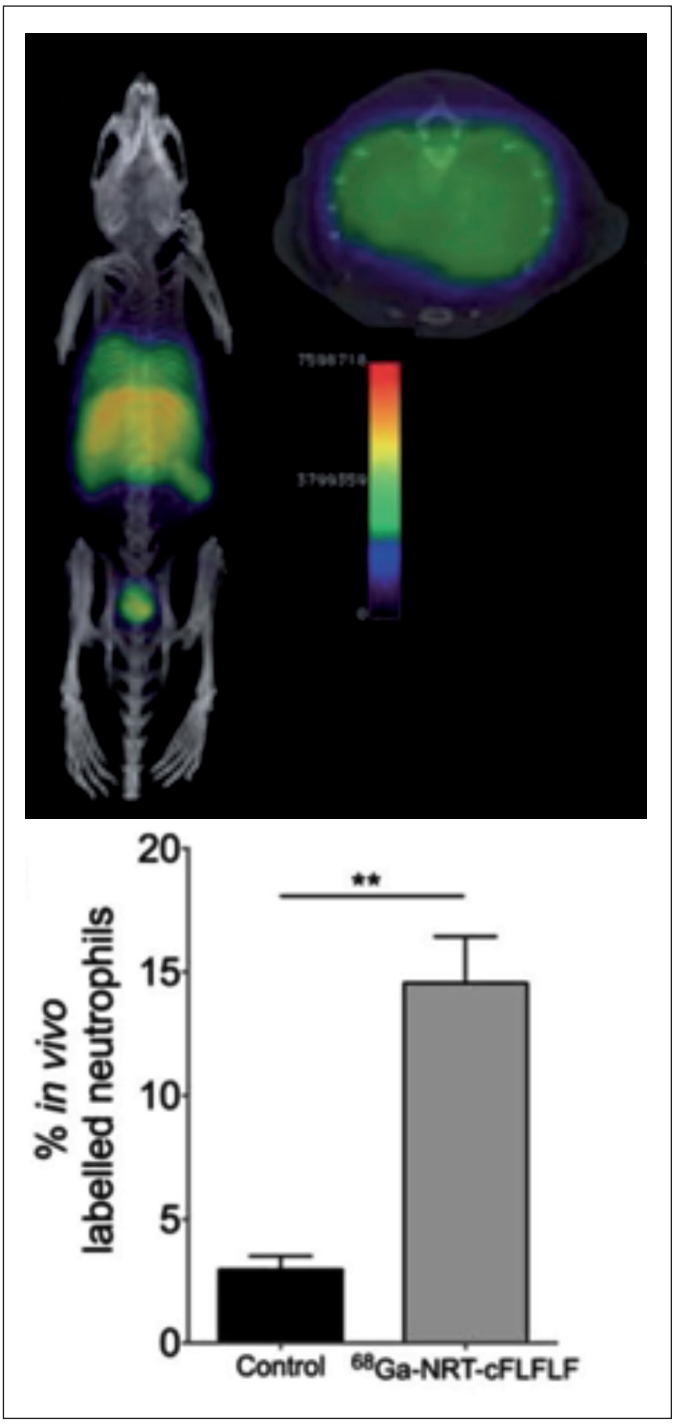
MOLECULAR IMAGING WITH NANOPARTICLES: SPECIFIC IN VIVO DETECTION OF NEUTROPHILS

J. Pellico[1], A.V. Lechuga-Vieco[1], E. Almarza[2,3], A. Hidalgo[1,4], C. Mesa-Nuñez[2,3], I. Fernández-Barahona[1,2,5], J.A. Quintana[1], J. Bueren[2,3], J.A. Enríquez[1], J. Ruiz-Cabello[6,7] and F. Herranz[1,5]

[1]Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC) and Centro de Investigación Biomédica en Red de Enfermedades Respiratorias (CIBERES), Madrid, Spain. [2]Division of Hematopoietic Innovative Therapies, Centro de Investigaciones Energéticas Medioambientales y Tecnológicas/Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Madrid, Spain. [3]IIS-Fundación Jiménez Díaz, Madrid, Spain. [4]Institute for Cardiovascular Prevention, Ludwig-Maximilians University, Munich, Germany. [5]NanoMedMol, Instituto de Química Médica, Consejo Superior de Investigaciones Científicas (CSIC), Madrid, Spain. [6]Universidad Complutense de Madrid, Madrid, Spain. [7]CIC biomaGUNE, Donostia-San Sebastián & Ikerbasque, Basque Foundation for Science, Bilbao, Spain.



Introduction: Non-invasive quantitative detection of lung inflammation is highly desirable for assessing pathogenic processes in the lung. Inflammatory-cell activation is currently assessed by combining anatomical imaging with information obtained from invasive lung biopsy, histopathology and bronchoalveolar lavages. This time-consuming approach explains the numerous attempts to produce a reliable probe for non-invasive in vivo diagnosis. Neutrophils are an essential part of the inflammatory cascade. However, neutrophil invasion can cause major tissue damage. There is mounting evidence on the role of neutrophils in the development of pulmonary hypertension. From the importance of neutrophil extracellular traps (NETs) in pulmonary arterial hypertension to the generation of reactive oxygen species or as a diagnosis tool of the disease.

Objectives: Our aim was to develop a multifunctional nanoparticle able to specifically detect in vivo the presence of neutrophils.

Methods: We synthesised 68Ga core-doped nanoparticles as a new kind of multimodal nano-radiotracer. These nanoparticles were functionalised with a neutrophil-specific peptide. LPS-induced lung inflammation was used as a model. Intravenous injection of the tracer was further followed by PET imaging and histological analysis.

Conclusions: This new nano-radiotracer[1] has been used for non-invasive in vivo detection of acute and chronic inflammation in the lungs with very high in vivo labelling efficiency, i.e. a large percentage of labelled neutrophils. Furthermore, we demonstrated that the tracer is neutrophil-specific and yields images of neutrophil recruitment of unprecedented quality[2].

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ACTIVATION OF KV7 CHANNELS AS A NOVEL MECHANISM FOR NO/CGMP-INDUCED PULMONARY VASODILATION

G. Mondéjar-Parreno[1,2,3], B. Barreira[1,2,3], M. Callejo[1,2,3], D. Morales-Cano[1,2,3], S. Esquivel[1,2,3], A. de la Cruz[4], L. Moreno[1,2,3], C. Valenzuela[4], F. Pérez-Vizcaíno[1,2,3] and A. Cogolludo[1,2,3]

[1]Deparment of Pharmacology and Toxicology, Universidad Complutense de Madrid, Spain. [2]CIBER de Enfermedades Respiratorias (CIBERES), Spain. [3]Instituto de Investigación Sanitaria Gregorio Marañón (IiSGM), Spain. [4]Instituto de Investigaciones Biomédicas Alberto Sols CSIC-Universidad Autónoma de Madrid, Spain.

Introduction: The nitric oxide-NO/cGMP pathway plays a key role in the control of pulmonary arterial (PA) tone and drugs activating this pathway (phosphodiesterase V inhibitors and soluble guanylate cyclase-sGC stimulators) are important therapeutic options to treat pulmonary arterial hypertension. Recent studies indicate that Kv7 channels are main regulators of vascular tone in several blood vessels, including the pulmonary circulation. However, their possible modulation by the NO/cGMP pathway remains unknown.
Objectives: We aimed to analyze the possible contribution of Kv7 channels to the pulmonary vasodilatation induced by NO donors and the sGC stimulator riociguat.
Methods: Membrane potential and K currents were recorded using the whole cell configuration of the patch-clamp technique in male Wistar rat or human PA smooth muscle cells (PASMC). Currents were evoked by application of voltage ramps. PA were mounted on a wire myograph for vascular reactivity studies. The effects induced by NO donors (DEA-NO and sodium nitroprusside-SNP) and riociguat were analyzed. The role of Kv7 channels was characterized by using selective Kv7 blockers (XE991 or linopirdine). In some experiments Kv1.5 channels were inhibited by the application of long (4s) depolarizing steps or using a selective inhibitor (DPO-1).
Results: In PASMC, DEA-NO and SNP hyperpolarized the membrane potential and induced a bimodal effect on K currents (augmenting the current between -40 to -10 mV and decreasing it at more depolarized potentials) when a 1s ramp was applied. The hyperpolarization and the enhancement of the current were suppressed in the presence of Kv7 channel inhibitors but preserved by DPO-1 or after 4s depolarizing pulses. The increase of the current and the hyperpolarization were also prevented by the sGC inhibitor ODQ and mimicked by Riociguat. Likewise, the electrophysiological effects induced by riociguat were prevented by XE991. In line with these data, PA relaxation induced by DEA-NO or riociguat was attenuated by Kv7 inhibitors.
Conclusions: Our study provides the first evidence that Kv7 channels contribute to the pulmonary vasodilation induced by the NO/cGMP pathway. Our data may be of great interest since it identifies a new mechanism of action of drugs used in the treatment of pulmonary arterial hypertension.
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THE ABILITY OF EXOSOMES DERIVED FROM MESENCHYMAL STEM CELLS TO LIMIT PULMONARY VASCULAR DYSFUNCTION IS ENHANCED BY HYPOXIC PRECONDITIONING

S. Esquivel-Ruiz[1,2,3], M. Álvarez-Fuente[4], F. Royo[5], B. Barreira[1,2,3], A. González-Murillo[6], J.M. Falcón-Pérez[5], M. Ramírez-Orellana[6], M. Jesús del Cerro[4], A. Cogolludo[1,2,3], F. Pérez-Vizcaíno[1,2,3] and L. Moreno[1,2,3]

[1]Department of Pharmacology and Toxicology, School of Medicine, Universidad Complutense de Madrid, Spain. [2]CIBER de Enfermedades Respiratorias (CIBERES), Madrid, Spain. [3]Instituto de Investigación Sanitaria Gregorio Marañón (IISGM), Spain. [4]Servicio de Cardiología Pediátrica, Hospital Universitario Ramón y Cajal, Madrid, Spain. [5]Exosomes Lab., CIC bioGUNE, CIBERehd, Derio, Bizkaia, Spain. [6]Unidad de Terapia Celular, Hospital Niño Jesús, Instituto de Investigación Sanitaria La Princesa, Madrid, Spain.

Introduction: Preclinical studies have demonstrated promising results using mesenchymal stem cells (MSCs) for the treatment of inflammatory lung diseases, including acute respiratory distress syndrome (ARDS), pulmonary arterial hypertension (PAH) or bronchopulmonary dysplasia (BPD). However, recent evidences suggest that their therapeutic activity is mainly mediated by paracrine mediators, most notably exosomes, rather than cell engraftment. Hypoxic preconditioning is thought to enhance the therapeutic potential of MSCs.
Objectives: In this work, we aimed to analyse whether MSCs-derived exosomes are able to prevent the pulmonary vascular dysfunction induced by lipopolysaccharide (LPS) and to enhance their effectiveness by the use of hypoxia-preconditioning.
Methods: EVs released by umbilical cord blood-derived MSCs were obtained by differential ultracentrifugation. Rat pulmonary arteries (PA) were treated overnight with LPS in the absence or presence of EVs. IL-6 levels were determined by ELISA and nitrite accumulation by Griess assay. Contractile responses were analysed in a wire myograph in the absence or presence of DETCA (SOD inhibitor).
Results: Exposure to LPS significantly increased IL-6 release, produced hyporresponsiveness to phenylephrine (due to increased iNOS activity), inhibited hypoxic pulmonary vasoconstriction (HPV), induced endothelial dysfunction and potentiated the contractile effects induced by serotonin in isolated PA. Treatment with MSC-derived exosomes had no effect in the iNOS activity and IL-6 production. In contrast, hypoxic exosomes significantly prevented the impairment of HPV and the hyperresponsiveness to serotonin. In addition, hypoxic exosomes attenuated the endothelial dysfunction induced by either LPS or DETCA.
Conclusions: Our data show that hypoxia-preconditioned MSCs-derived exosomes prevent the development of pulmonary vascular dysfunction induced by LPS and high oxidative stress in isolated PA. These findings suggest that hypoxic preconditioning increases the therapeutic potential of MSCs-derived exosomes and may represent a new therapeutic approach in pulmonary vascular diseases associated with inflammation.

IS IRON DEFICIENCY MODULATING PHYSICAL ACTIVITY IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE?

C. Martín-Ontiyuelo[1], A. Rodo-Pin[1], A. Sancho-Muñoz[1], M. Admetllo[1], J.M. Martínez Llorens[1], L. Molina Ferragut[2], E. Barreiro[1], J. Gea Guiral[1] and D.A. Rodríguez Chiaradia[1]

[1]Pulmonology Department, Hospital del Mar-IMIM, Parc de Salut Mar, Universitat Pompeu Fabra, Barcelona, Spain and CIBER de Enfermedades Respiratorias (CIBERES), Madrid, Spain. [2]Cardiology Department, Hospital del Mar-IMIM, Parc de Salut Mar, Universitat Pompeu Fabra, Barcelona, Spain.

Introduction: There is evidence that iron plays a key role in the adequate functioning of skeletal muscle. While it has been demonstrated

that iron deficiency (ID) affects exercise tolerance and response to exercise training in patients with chronic obstructive pulmonary disease (COPD)[1], the impact on daily physical activities (DPA) remains unknown.
Objectives: To evaluate if iron deficiency modulates physical activity in COPD.
Methods: Seventeen COPD patients with ID (ferritin < 100 ng/mL or ferritin 100-299 ng/mL with a transferrin saturation < 20%) and 17 COPD patients without this abnormality, matched for age and disease severity (control group) were enrolled to the study. The primary outcome was the level of DPA assessed by accelerometers.
Conclusions: Patients had (mean [SD] 64) (7) yrs. and were mostly male (70%), and former smokers (70%). Their FEV1 was 37(11) % pred., DLco was 50 (16) % pred. and PaO_2 reached 70 (8) mmHg. DPA and the number of steps per day were lower in ID COPD patients compared with controls (PAL [physical activity level] 1.37 vs. 1.55, $P = 0.025$; and 3,628 vs. 7,072 steps/day, $P = 0.038$ respectively). In contrast, the time spent sitting was higher in the former patients (7 vs. 4 hours/day, $P = 0.042$), whereas the time doing moderate to vigorous activity (> 3 MET) showed a trend to be lower (and 75 vs. 120 minutes/week, $P = 0.181$). We concluded that the presence of iron deficiency was associated with reduced daily physical activities in COPD patients. Further studies are needed to evaluate iron reposition and their impact on the level of physical activity in these patients.
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LABELED NANOVECTORS ENGINEERED FOR LUNG TARGETING

S. Carregal Romero[1,2], K.R. Pulagam[3], P. Ramos-Cabrer[4,5], J. Llop[3] and J. Ruiz Cabello[1,2,6]

[1]Molecular and Functional Biomarkers Group, CIC biomaGUNE, Donostia-San Sebastián, Spain. [2]CIBER de Enfermedades Respiratorias (CIBERES), Madrid, Spain. [3]Radiochemistry and Nuclear Imaging Lab., CIC biomaGUNE, Donostia-San Sebastián, Spain. [4]Magnetic Resonance Imaging Lab., CIC biomaGUNE, Donostia-San Sebastián, Spain. [5]Ikerbasque, Basque Foundation for Science, Bilbao, Spain. [6]Universidad Complutense de Madrid, Madrid, Spain.

Introduction: Drug delivery through the lungs for the treatment of respiratory diseases is advantageous with respect to other administration routes because it is applied directly to the site of action, it is noninvasive and offers a large surface area for drug absorption. In this context, colloidal nanovectors are often required to improve the solubility of certain drugs and for sustained drug release. Moreover, they can carry tags for lung imaging. In our work we will introduce colloidal particles that have demonstrated in test tube, in vitro and in vivo experiments an efficient capacity for controlled drug delivery and discuss about the challenges to be used for inhalation and lung delivery.
Objectives: Synthesize nanogels, liposomes and magnetic nanoparticles with theranostic characteristics and evaluate their efficiency for the sustained delivery of small hydrophobic drugs and macromolecules such as proteins or nucleic acids to the lungs.
Methods: Free radical vinyl polymerization will be used for the synthesis of stimuli responsive nanogels. Liposomes will be prepared using the lipid film rehydratation and extrusion method and the iron oxide based nanoparticles will be prepared with microwave assisted hydrothermal protocols. Drugs will be encapsulated by chemical bonding and physical caging. Drug delivery will be studied by optical spectroscopy and liquid chromatography.
Conclusions: Colloidal synthesis represents a toolbox for building drug and contrast agents carriers with unlimited possibilities. However the correct evaluation of toxicity and efficiency is key for their real application in clinics. In our work we will compare the efficiency of different nanovectors for treating drug diseases such as lung hypertension.

LUNG MECHANICAL STRETCH TRIGGERS A SENESCENCE-LIKE RESPONSE

J. Blázquez-Prieto[1,2], C. Huidobro Fernández[2,3], I. López Alonso[2,3], L. Amado Rodríguez[2,3,4], C. López Martínez[1,5] and G.Muñiz Albaiceta[1,2,3,4,5]

[1]Departamento de Biología Funcional, Universidad de Oviedo, Oviedo, Spain. [2]CIBER de Enfermedades Respiratorias (CIBERES), Madrid, Spain. [3]Instituto de Investigación Sanitaria del Principado de Asturias, Oviedo, Spain. [4]Unidad de Cuidados Intensivos Cardiológicos, Hospital Universitario Central de Asturias, Oviedo, Spain. [5]Instituto Universitario de Oncología del Principado de Asturias, Oviedo, Spain.

Introduction: Senescence is currently understood not only as a long-term consequence of cell stress, but also as part of the acute response to injury. Abnormalities in the interaction between DNA and nuclear envelope may trigger senescence. As the nuclear envelope has recently been involved in mechanotransduction, we hypothesized that mechanical stretch could activate senescence-related cell pathways, aimed to minimize tissue damage.
Objectives: Our objectives were to study the activation of senescence-related molecular pathways after lung stretch caused by mechanical ventilation, and to clarify the short-term consequences of this activation.
Methods: Lung injury was induced by intratracheal HCl instillation and mechanical ventilation in C57Bl/6 mice. After 135 minutes, animals were sacrificed and lungs harvested. Lung damage was assessed in histological sections, and neutrophilic infiltration and Il6 expression were quantified as markers of inflammation. Senescence was characterized by appearance of Macro-H2A-positive heterochromatin foci and expression of p53 (Tp53), p16 (Cdkn2a) and p21 (Cdkn1a). DNA damage and heterochromatin abundance were quantified by western blots against γ-H2AX and HP1-α respectively. Senescence-associated heterochromatin foci were also assessed in lung sections from patients who died after mechanical ventilation and non-ventilated controls. To clarify the role of senescence in lung damage, mice treated with lopinavir-ritonavir (that ameliorates mechanical stretch within the nuclear envelope) or vehicle were compared after lung damage and mechanical ventilation. Similarly, animals lacking Cdkn1a (p21) and their wildtype counterparts were studied to clarify the role of this factor.
Results: Acid instillation and mechanical ventilation caused lung injury, with an increase in histological scores and apoptotic cell counts. Interestingly, both hits, but not acid instillation alone was related to the appearance of senescence-associated heterochromatin foci. Among the canonical markers of senescence, only Cdkn1a (p21) increased with injury. Samples from mechanically ventilated patients showed also heterochromatin foci. The mechanical stretch caused alterations in the nuclear envelope, namely an increase in Lamin-A/Lamin-B ratio, an increase in the DNA damage marker γ-H2AX and in HP1-α. Treatment with lopinavir-ritonavir decreased lung injury and apoptosis in spite of no changes in DNA damage. However, this treatment dampened all the observed changes in chromatin structure (senescence-associated heterochromatin foci and abundance of HP1-α) and markedly increased the expression of Cdkn1a (p21). Finally, lung injury and apoptosis were markedly increased after acid instillation and mechanical ventilation in Cdkn1a-/- mice, compared to their wildtype counterparts.
Conclusions: Mechanical stretch triggers senescence in injured lungs. This response is aimed to limit epithelial apoptosis and minimize injury.