92. The ageing pulmonary interstitium

P781 Ambrisentan attenuates lung and heart injury in a rat model of bronchopulmonary dysplasia
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The selective endothelin receptor type A antagonist ambrisentan may be a novel therapeutic agent in neonatal chronic lung disease by blocking the adverse effects of the potent vasoconstrictor endothelin-1, including pulmonary arterial hypertension (PAH)-induced right ventricular hypertrophy (RVH). The cardiopulmonary effects of ambrisentan were studied in neonatal rats with hyperoxia-induced lung injury. Ambrisentan treatment was investigated in 2 models of experimental BPD: a prophylactic model, in which pups were continuously exposed to hyperoxia and treated daily with either saline or ambrisentan (20 mg/kg body weight/day; injected subcutaneously), and an injury-recovery model, in which pups were exposed to hyperoxia for 9 days, followed by 9 days of recovery in room-air and treatment with ambrisentan started on day 6 of oxygen exposure and continued during the recovery period. In the prophylactic model treatment with ambrisentan improved survival ($p < 0.01$) by reducing lung fibrin deposition (3-fold, $p < 0.001$), alveolar septum thickness (1.7-fold, $p < 0.001$) and medical wall thickness of small arterioles as a marker for PAH (1.7-fold, $p < 0.001$), and preventing associated RVH ($p < 0.001$). Treatment with ambrisentan did not have beneficial effects on alveolar enlargement, vascularization, the pulmonary influx of macrophages and neutrophils, and the mRNA expression of procoagulant and inflammatory markers. In the injury-recovery model treatment with ambrisentan attenuated PAH and RVH ($p < 0.001$), demonstrating that established PAH-induced RVH is still reversible in the neonatal period. Beneficial effects on reduced pulmonary vascularization and alveolarization were absent.

P782 Protease-activated receptor-2 triggers epithelial to mesenchymal transition: Potential relevance in pulmonary fibrosis
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Idiopathic pulmonary fibrosis (IPF) constitutes the most devastating form of fibrotic lung disorders. The destructive fibroblast/foci characteristics of IPF originate, at least partly, via epithelial to mesenchymal transition (EMT). The extracellular signals and cellular receptors triggering EMT in IPF remain incompletely understood however. Recently, we showed that protease-activated receptor-2 (PAR-2), a transmembrane G-protein-coupled receptor expressed ubiquitously in the lung, is an essential player in fibrotic lung disorders by directly targeting fibroblasts. Here, we explore the role of PAR-2 on epithelial cells by focusing on PAR-2-induced EMT in pulmonary fibrosis. Immunostaining of lung biopsies of IPF patients showed prominent PAR-2 expression by fibroblasts and epithelial cells overlying fibrotic foci. Double stainings indicated that PAR-2 co-localized on cells expressing both epithelial (cytokeratins) and mesenchymal (vimentin) markers, indeed suggesting a role of PAR-2 in EMT. In vitro experiments showed that PAR-2 stimulation induced a fibroblast-like morphology in type II lung epithelial cells, the expression of the myofibroblast markers vimentin and α-SMA, and the secretion of collagen. Interestingly, PAR-2 stimulation triggered β-catenin accumulation and translocation to the nucleus. In conclusion, PAR-2 triggers EMT of epithelial cells and PAR-2-dependent activation of the β-catenin/WNT signaling pathway is probably the main driver of PAR-2-induced EMT. Overall our data thus suggest that inhibition of the PAR-WNT axis may be a clinically relevant treatment option in IPF but also in other disorders in which EMT is essential.

P783 Disruption of Nrf2 enhances susceptibility to pulmonary fibrosis induced by bleomycin in mice
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Heat shock protein (HSP) 47, a collagen-specific molecular chaperone, is involved in the processing and/or secretion of procollagen. The aim of this study was comparative analysis of the diagnostic values of serum HSP47. Krebs von den Lungen-6 (KL-6), surfactant protein (SP-A), SP-D and lactate dehydrogenase (LDH) levels for rapidly progressive interstitial pneumonia. Subjects comprised 27 patients with rapidly progressive interstitial pneumonia, 12 with cryptogenic organizing pneumonia (COP), 19 with idiopathic interstitial pneumonia (UIP), 16 with idiopathic nonspecific interstitial pneumonia (NSIP), 11 with collagen vascular disease-associated UIP, 11 with collagen vascular disease-associated NSIP, and 18 healthy adult volunteers. Serum levels of HSP47 in patients with rapidly progressive interstitial pneumonia were significantly higher than those in patients with COP, idiopathic UIP, idiopathic NSIP, collagen vascular disease-associated UIP, collagen vascular disease-associated NSIP and healthy volunteers. Receiver operating characteristic curves revealed that HSP47 was superior to the other markers. The cut-off level for HSP47 that resulted in the highest diagnostic accuracy was 896.9 pg/ml. The sensitivity, specificity, and diagnostic accuracy were 92.6%, 100%, and 98.2%, respectively. These results suggest that of the markers studied, HSP47 is the best serum marker for rapidly progressive interstitial pneumonia.
The fibroblastic foci were induced by bleomycin at a dosage of 90 mg/kg cell populations on days 0, 3, 7, 10, 14, 21, and 28. Body weight on day 0, and the bronchoalveolar lavage (BAL) fluid examined for bleomycin was administered intravenously to the mice at a dosage of 80 mg/kg and 90 mg/kg body weight on day 0, and the fibroblastic foci were assessed histologically by Ashcroft score determined in the lung tissues on day 28. Furthermore, bleomycin was administered intravenously to the mice at a dosage of 0, 70, 80, and 90 mg/kg body weight on day 0, and the bronchoalveolar lavage (BAL) fluid examined for cell populations on days 0, 3, 7, 10, 14, 21, and 28. Results: The fibroblastic foci were induced by bleomycin at a dosage of 90 mg/kg body weight in the lung tissues on day 28 in Nrf2+/− mice. In contrast, the fibroblastic foci were induced by bleomycin at a dosage of 70 mg/kg body weight in Nrf2−/− mice. The total number of cells and macrophages in the BAL fluid were significantly increased from day 7 after bleomycin administered in both Nrf2+/− and Nrf2−/− mice. The increased cells number were significantly greater in Nrf2−/− mice than in Nrf2+/− mice. Conclusions: These findings suggest that Nrf2 might be an important genetic factor in the determination of susceptibility to bleomycin induced pulmonary fibrosis by regulating the macrophages defense mechanisms.

**P786**

**Work of inflation is the best correlate to lung fibrosis induced by bleomycin in mice**

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**Introduction:** Bleomycin (BLM) induces a transient lung fibrosis that has been used to investigate mechanisms related to lung fibrogenesis. Our aim was to determine the method for assessing lung function that provided the strongest correlation to the lung fibrosis.

**Methods:** BLM (2 U/kg) or saline was intratracheally microsprayed to male, C57BL/6 mice under isoflurane. Lung function was assessed using the flexus system and after euthanasia, lungs were inflated in situ under a constant pressure of 25 cm H2O starting 1–35 d after BLM administration (n = 8). Right and left lung sections, stained with Masson’s trichrome, were graded for fibrosis (0–4, i.e. no fibrosis to severe) and the scores were combined (0–8).

**Results:** Lung fibrosis scores were progressive 1–14 d after BLM challenge. From day 14, the fibrosis scores did not significantly change. Lung function changes were more subtle and no significant changes in resistance or elastance were observed after day 21 post-BLM. The only significant change occurred in work of inflation (Wol).

**Conclusion:** Scores for lung fibrosis are the robust indicator of BLM-induced changes to the lung. Although changes to lung function were obvious, Wol provided a greatest level of precision to detect significant changes.

**P787**

**Levels of cytokines and chemokines in BAL fluid in patients with idiopathic interstitial pneumonitis and collagen vascular disease associated interstitial pneumonitis**

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Inflammatory cytokines and chemokines have been reported to play important roles in the pathogenesis of interstitial lung diseases. However, their individual roles in idiopathic interstitial pneumonitis (IP) and in other types of interstitial pneumonitis (IP), including collagen vascular disease associated interstitial pneumonitis (CVID-IP), remain unknown. BAL fluid levels of IL-1β, -2, -4, -5, -6, -7,-8, -10, -12, -13, -17, G-CSF, IFN-γ, MCP-1, MIP-1α, and TNF-α were measured using a bead suspension array in 8 patients (5 men, 3 women; mean age, 60.0 ± 9.9 years) with idiopathic nonspecific interstitial pneumonitis (NSIP), 6 patients (3 men, 3 women; mean age, 69.0 ± 4.8 years) with idiopathic usual interstitial pneumonitis (UIP), 3 patients (2 men, 1 woman; mean age, 66.3 ± 5.5 years) with rheumatoid arthritis (RA), and 3 patients (1 man, 2 women; mean age, 52.3 ± 14.3 years) with dermatomyositis (DM) in CVID-IP, as well as in 13 patients (2 men, 11 women; mean age, 58.1 ± 17.2 years) sarcoidosis, as a disease control. Levels of IL-7 were highest for DM (19.0 ± 6.8 pg/ml), compared with other IPs (9.6 ± 3.7 pg/ml for UIP, 6.1 ± 3.7 pg/ml for NSIP, 7.0 ± 6.9 pg/ml for RA) and sarcoidosis (4.2 ± 2.5 pg/ml). On the other hand, levels of TNF-α were highest for RA (27.8 ± 37.0 pg/ml), compared with other IPs (2.3 ± 1.1 pg/ml in UIP, 1.3 ± 0.8 pg/ml in NSIP, 10.7 ± 9.3 pg/ml in DM) and sarcoidosis (5.9 ± 6.1 pg/ml).

Interestingly, levels of IL-17 were detectable only in RA (5.2 ± 5.0 pg/ml). Differences seen in the level of each cytokine and chemokine between patients with IP and CVID-IP might reflect the pathogenesis of the IP.
P788 Enhanced acute pulmonary inflammation and reduced fibrotic response in quartz-exposed p4/7phox-deficient mice
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Previous studies have shown that quartz (crystalline silica) provides a useful tool to study experimental fibrosis in rodents. In the present study, we have investigated the involvement of phagocyte-derived reactive oxygen species (ROS) in quartz-induced inflammatory and fibrotic responses. NADPH oxidase p4/7phox subunit deficient mice and their wild type counterparts were exposed to 100 mg/kg b.w quartz via a single pharyngeal aspiration. After 24 hours markers of myofibroblast and oxidative stress were investigated in bronchoalveolar lavage fluid (BALF) and lung tissue. Quartz elicited a strong acute inflammatory response, characterised by a remarkably similar pulmonary influx of neutrophils in both strains. Interestingly, however, luminescence multiplex analysis of BALF revealed stronger increases of interleukin (IL)-1β, IL-6, IL-17, keratinocyte-derived chemokine (KC), monocyte chemottractant protein-1 ( MCP-1) and granulocyte colony stimulating factor (G-CSF) in the knockout mice compared to wild type animals. Differences in IL-4, IL-10, IL-13 and tumour necrosis factor-alpha (TNF-α) were not detectable. In contrast, pulmonary mRNA levels of the oxidative stress markers γ-glutamyl cysteine synthetase (γ-GCS) and heme oxygenase-1 (HO-1) were significantly enhanced only in the wild type mice in response to quartz-treatment. Three months after quartz treatment, significantly less fibrosis occurred in the lungs of knockout mice, as indicated by hydroxyproline content and Masson's trichrome staining.

These data show that impairment of NADPH oxidase increases acute inflammatory responses, whereas it reduces oxidative stress and fibrosis in the lungs of quartz-exposed mice.

P789 Mucaricn receptor stimulation differentially regulates extracellular matrix gene expression in lung fibroblasts
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Airway fibrosis is a characteristic feature of both asthma and COPD, in which fibroblasts are importantly involved. Increased activity of the cholinergic system may contribute to airway fibrosis stimulation as muscarinic receptor stimulation has been shown to enhance collagen deposition by fibroblasts. The effects of muscarinic receptor stimulation on the expression profile of other extracellular matrix (ECM) proteins, however, remains to be established. To assess the effects of muscarinic receptor stimulation on ECM gene expression, primary human lung fibroblasts were exposed to increasing concentrations of the muscarinic receptor agonist carbachol (0.01-100 μM) for 3,6 and 9 days. Real time PCR analysis demonstrated a significant concentration-dependent upregulation of α-smooth muscle actin as well as collagen-I, -III and -IV and fibronectin in a time dependent manner. Little or no effects were observed on the expression of biglycan, versican or αtative PCR. The results demonstrate that methacholine concentration-dependently enhanced expression of the laminin α1 chain, whereas expression of the decorin α1 chain was decreased. No effects of methacholine were observed on the gene expression of collagen I, collagen III, fibronectin, biglycan, versican or laminin α2, β1 and γ1 chains. In the presence of TGF-β1, methacholine (10 μM) enhanced expression of fibroblast growth factor type I (FGF-1), but no additional effects of methacholine were observed on the expression of the other ECM genes investigated. Collectively, these results indicate that muscarinic receptor stimulation selectively changes the expression of specific ECM genes, which may contribute to the airway remodelling as observed in asthma and COPD.

P790 Collagen V and decorin is involved in systemic sclerosis pulmonary fibrosis
Patricia Martin1, Ana Paula Velosa1, Adriana Santos1, Solange Carrasco1, Angela Santos2, Edwin Parra2, Claudia Goldenstein-Schah second1

Systemic sclerosis (SSc) is characterized by vascularopathy, inflammation, autoimmunity and fibrosis. Collagen V (COLV) is involved in SSc in relation to the presence of the fibrogenic growth factor TGF-β1 (4 ng/ml), after which ECM gene expression was determined by quantitative PCR. The results demonstrate that methacholine concentration-dependently enhanced expression of the laminin α1 chain, whereas expression of the decorin α1 chain was decreased. No effects of methacholine were observed on the gene expression of collagen I, collagen III, fibronectin, biglycan, versican or laminin α2, β1 and γ1 chains. In the presence of TGF-β1, methacholine (10 μM) enhanced expression of fibroblast growth factor type I (FGF-1), but no additional effects of methacholine were observed on the expression of the other ECM genes investigated. Collectively, these results indicate that muscarinic receptor stimulation selectively changes the expression of specific ECM genes, which may contribute to the airway remodelling as observed in asthma and COPD.

P791 The effect of erythropoietin (EPO) on cyclorcycyanogenase-2 (COX-2) and cyclochrome-c (CYT-c) in the b1omyosin (B1MY)-induced pulmonary fibrosis (PF) in rats
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Purpose: The enzymes cycloxyge COX-2 and CYT-c are known to be a part of the fibrotic pathway. EPO is a multiple functional cytokine with anti-inflammatory and anti-apoptotic properties. Aim of this study was to investigate the role of EPO on the expression of both enzymes in BLM-induced PF in rats.

Methods: Fifty Wistar rats (300g) were divided into five groups of animals each: 1) control animals; 2) intratracheal (i.t.) and intraperitoneal (i.p.) injection of bleomycin (BLM) (2.5mg/kg) i.t injection, 3) BLM hydrochlo ride (7.5mg/kg) i.t injection followed by EPO i.p injection (2000 iu/kg), 5) saline (0.5ml/kg) i.t injection followed by EPO i.p injection (2000 iu/kg). All rats were sacrificed after 14 days. Immunohistological examination was performed for the expression of COX-2 and CYT-c.A scale of 4 grades was used for the evaluation of the results: 0.25 % (A), 25-50% (B), 50-75% (C), 75-100% (D).

Results: In groups1 and 2, both COX-2 and CYT-c were expressed in the grade A (80%) and in the grade B (20%). In group 3, COX-2 was expressed in the high grades B (20%), C (60%) and D (20%), and CYT-c only in the two higher grades (C 70%) and D (30%). In group 4, both enzymes were expressed only in the low grades A (80% and 70%) respectively and B (20% and 30% respectively). The expression of COX-2 and CYT-c took place in the high grades for BLM group and in the lower grades for BLM+EPO group (p<0.01 and p<0.05 respectively). Conclusions: BLM induction followed by EPO resulted in significant lower expression of COX-2 and CYT-c compared with BLM group. The protective mechanisms of EPO on PF must be further clarified.

P792 Microparticles-associated tissue factor activity is increased in bronchoalveolar lavage of patients with pulmonary fibrosis and correlates with functional impairment
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Background: The activation of the coagulation cascade plays a role in the progression of pulmonary fibrosis. Furthermore, the generation of inflammatory and pro-fibrotic mediators by activated platelet aggregates and platelet derived microparticles (MP) may contribute to airway fibrosis stimulation as muscarinic receptor stimulation has been shown to enhance collagen deposition by fibroblasts. The effects of muscarinic receptor stimulation on the expression profile of other extracellular matrix (ECM) proteins, however, remains to be established. To assess the effects of muscarinic receptor stimulation on ECM gene expression, primary human lung fibroblasts were exposed to increasing concentrations of the muscarinic receptor agonist carbachol (0.01-100 μM) for 3,6 and 9 days. Real time PCR analysis demonstrated a significant concentration-dependent upregulation of α-smooth muscle actin as well as collagen-I, -III and -IV and fibronectin in a time dependent manner. Little or no effects were observed on the expression of biglycan, versican or αtative PCR. The results demonstrate that methacholine concentration-dependently enhanced expression of the laminin α1 chain, whereas expression of the decorin α1 chain was decreased. No effects of methacholine were observed on the gene expression of collagen I, collagen III, fibronectin, biglycan, versican or laminin α2, β1 and γ1 chains. In the presence of TGF-β1, methacholine (10 μM) enhanced expression of fibroblast growth factor type I (FGF-1), but no additional effects of methacholine were observed on the expression of the other ECM genes investigated. Collectively, these results indicate that muscarinic receptor stimulation selectively changes the expression of specific ECM genes, which may contribute to the airway remodelling as observed in asthma and COPD.

P793 Study of tryptophanbiotin in idiopathic pulmonary fibrosis and COPD
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Introduction: Tryptophanbiotin (BHT) is an essential cofactor for the activity

Results: COLV fibers was distorted and thickened in SSc lung tissue compared to thin fibers of controls. Decorin was distributed around COLV fibrils in the bronchovascular interstitium and vascular walls. Histomorphometric analysis of SSc demonstrated increased expression of COLV and decorin (p<0.01) when compared to control. Immunohotting detected an increased high molecular weight COLV fraction in SSc (p<0.02).

Conclusion: Over expression and unusual organization of COLV fibers with bio-chemical changes associated to increased decorin indicates that matrix signalization pathway is involved in COLV fibrillogenesis process in SSc pulmonary fibrosis.

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of nitric oxide synthase enzyme (eNOS). Deficiency of BH4 induced by oxidative stress could produce eNOS uncoupling and contribute to pulmonary damage.

**Objective:** To study the role of endogenous BH4 in patients with stable idiopathic pulmonary fibrosis (IPF) and chronic obstructive pulmonary disease (COPD).

**Materials and methods:** Twenty eight patients (15 IPF, 13 COPD; 61 (13) years and 9 healthy controls were studied. Lung function tests (spirometry, plethysmograhy and lung diffusion capacity), HRCT (High Resolution Computed Tomography) lung scan and 6-min. walk test were performed in all patients. Blood neutrophilic plasma BH4 (reverse-phase high-performance liquid chromatograph: RP-HPLC) with C18 column, hemoglobin, fibrinogen, and CRP were also measured.

**Results:** BH4 levels were significantly reduced in IPF (1.32 (0.16), and COPD (1.44 (0.23)) patients versus controls (2.42 (0.29)). There were no differences in BH4 levels between either IPF and COPD or bronchitis and eosinophilia phenotypes. BH4 levels were not related with parameters of lung function, radiological extension, inflammatory markers or smoking severity. In patients with COPD, BH4 levels were related with the number of previous exacerbations.

**Conclusions:** Plasma BH4 levels are reduced in IPF and COPD, which may be of potential value as a future biomarker of oxidative stress related diseases. Supported by grant SVN 2010 and CIBERES (CB06/00027)

**P794 Increase of nitrosative stress in patients with eosinophilic pneumonia**

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**Background:** Exhaled nitric oxide (NO) production is increased in asthma and may be involved in the pathogenesis.

**Objective:** To investigate whether NO production is increased in eosinophilic pneumonia (EP).

**Methods:** Exhaled NO was measured in healthy subjects and in patients with eosinophilic pneumonia including idiopathic pulmonary fibrosis (IPF), cryptogenic organizing pneumonia, hypersensitivity pneumonitis, sarcoidosis and EP. iNOS expression and 3-NT positive cells were observed in the EP group compared to the healthy subject and IPF patient.

**Results:** The Calv levels in the EP group were significantly higher than those in the healthy subjects and the other interstitial pneumonia groups as well as the fractional exhaled NO (FeNO) levels. More iNOS and 3-NT positive cells were observed in the EP group compared to the healthy subject and IPF patient. The Calv levels had significant correlations with both iNOS (p < 0.05) and 3-NT positive cells (p < 0.01). Corticosteroid treatment significantly reduced both the iNOS and Calv levels (p < 0.01). The magnitude of reduction in the Calv levels had a significant correlation with the peripheral blood eosinophil counts (p < 0.05).

**Conclusion:** These results suggested that nitrosative stress was augmented in EP and may be involved in the pathogenesis.

**P795 Molecular mechanism of lung aging in senescence-accelerated mouse (SAM)**

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**The SAM strains are a collection of inbred mouse strains developed as models of accelerated aging, and include nine short-lived, the senescence-prone strains (SAMP) and three longer lived control strains designated the senescence-resistant strains (SAMR). The SAMR was suggested as a new murine model of aging lung. However, molecular mechanism of accelerated lung aging remains to be elucidated. By using quantitative real time RT-PCR and western blot, here we show that expression of FOXO, a forkhead transcription factor that acts downstream of the PTEN/PISK/Akt pathway and a key regulator of stress resistance, metabolism and aging, was significantly decreased in aged SAMP mice compared to normal aging control SAMR mice. The decreased expression of FOXO gene was correlated with elevation of reactive oxidative species (ROS) and thiobarbituric acid reactive substances (TBARS), reduced mRNA expression levels of superoxide dismutase (SOD2) and catalase, as well as greater mean linear intercept (MLI) in SAMP mice lungs. Based on these findings we concluded that reduced FOXO activity (SOD2) and catalase, as well as greater mean linear intercept (MLI) in SAMP mice. The decreased expression of FOXO gene was correlated with elevation of reactive oxidative species (ROS) and thiobarbituric acid reactive substances (TBARS), reduced mRNA expression levels of superoxide dismutase (SOD2) and catalase, as well as greater mean linear intercept (MLI) in SAMP mice lungs. Based on these findings we concluded that reduced FOXO activity (SOD2) and catalase, as well as greater mean linear intercept (MLI) in SAMP mice. Based on these findings we concluded that reduced FOXO activity (SOD2) and catalase, as well as greater mean linear intercept (MLI) in SAMP mice. Based on these findings we concluded that reduced FOXO activity (SOD2) and catalase, as well as greater mean linear intercept (MLI) in SAMP mice.
Consistent with emphysema, lung compliance was increased in *fro/fro* mice.

We found that heterozygous females were less affected than heterozygous males.

**Conclusion:** In contradiction with the current hypothesis, we have shown that nSMase2-deficient mice develop emphysema. We conclude that at least some levels of ceramide are necessary to ensure proper lung development.

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**P799**

Late intervention with a myeloperoxidase inhibitor prevents emphysema and small airway remodeling in the guinea pig

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Considerable evidence links both inflammation and oxidative stress to the pathogenesis of COPD. Myeloperoxidase (MPO), a neutrophil product, plays a major role in bacterial killing via production of the powerful oxidant, HOCl. However, oxidants generated by MPO can damage tissue and MPO exerts a variety of other effects that drive inflammation. We examined the effects of an MPO inhibitor, AZ11938920 on chronic (6 month) cigarette smoke-induced lesions in the guinea pig. One group of animals received compound from smoking day 1 (prophylactic arm), whereas another group was only treated after 3 months of smoke exposure (therapeutic arm). Analysis of lavage fluid showed that both treatments abolished smoke-induced increases in lavage inflammatory cells. Both treatments prevented smoke-induced increases in airspace size (emphysema) and small airway remodeling. Physiologically, both treatments largely reversed smoke-induced shifts of the pressure-volume and flow-volume curves and returned resistance to control values. Both treatments prevented muscularization of the small intrapulmonary arteries, but only partially ameliorated smoke-induced pulmonary hypertension. Immunohistochemical staining for the oxidation product, dityrosine, was increased in smoke-exposed animals and this effect was largely reversed by both treatments. We conclude that a myeloperoxidase inhibitor is able to prevent the development of emphysema and small airway remodeling and to partially protect against pulmonary hypertension, even when treatment starts after 3 months of smoke exposure. This protection appears to be related to prevention of oxidant damage and suppression of inflammation.