**369. Experimental pulmonary hypertension**

P3330

**Effect of interferon α preparations on IP10 and ET-1 release from human pulmonary artery smooth muscle cells**

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Pegylated (PEG) interferons (IFN), used to treat hepatitis C, are associated with lung toxicity and pulmonary hypertension. Pegylation increases stability of the IFN moiety and in vivo half-life, but reduces in vitro anti-viral activity. These effects are related to the size/shape/position of the PEG attachment. There are two marketed PEGIFNα preparations for hepatitis C; PEGIFNα2a and PEGIFNα2b, which are conjugated to 40 KDa and 12 KDa moieties respectively.

Endothelin-1 (ET-1) and IP10 are associated with lung inflammation and are induced by IFNs. We investigated the effect of IFNα preparations on ET-1 and IP10 release from human pulmonary artery smooth muscle (HPASM) cells. HPASM cells were treated with IFNs (0.33ng/mL to 30ng/mL). For ET-1, TNFα (10ng/mL) was added. IP10 and ET-1 immunoreactivity was measured by ELISA at 24h.

IFNα preparations induced IP10 with PEGIFNα2a being the weakest inducer (Fig. 1A; n=6; *p<0.05 by two way ANOVA). In TNFα treated cells, IFNα2a, IFNα2b and PEGIFNα2b induced ET-1 above baseline release (Fig. 1B; n=8; **p<0.05 by one way ANOVA vs control; #p<0.05 by two way ANOVA).

![Figure 1](image)

We conclude that IFNα preparations activate HPASM cells and this may contribute to the lung inflammation seen in some patients. PEGIFNα2a has the larger PEG moiety and induced least ET-1/IP10. Our results suggest ET-1/IP10 are important when considering mechanisms of pulmonary toxicity of IFNs.

P3331

**Could platelet-activating factor acetylhydrolase (PAF-AH) predict adverse event in pulmonary hypertension?**

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Chronic thromboembolic pulmonary hypertension (CTEPH) and pulmonary arterial hypertension (PAH) are threatened conditions mostly diagnosed at late stages. Inflammation could play a role in the pathogenesis. The need of new biomarkers, non-invasively measurable may help to improve the diagnosis and follow up. PAF-AH, a plasmatic enzyme, is a predictive risk factor for cardiovascular events.

In a prospective study, we have investigated a potential role of PAF-AH in...
predicting the outcome in PAH and CTEPH. Circulating PAH-AH activity has been measured in consecutive patients diagnosed with PAH (n=152) and CTEPH (n=115), at the time of right heart catheterization and compared to a control group of healthy subjects (n=115).

Circulating PAH-AH activity was lower in CTEPH and PAH patients compared to controls (37; 95% CI: 33-41; 41; 95% CI: 37-45; 54; 95% CI: 50-60 nmol/mL·min), p<0.0001. In PAH, PAH-AH activity is correlated to total cholesterol (r=0.29, p<0.0002) and to LDL-cholesterol (r=0.26, p=0.001). In CTEPH, PAH-AH activity is correlated to pulmonary vascular resistance (PVR; r=0.21, p=0.02) and to LDL-cholesterol (r=-0.22, p=0.01). In PAH, clinical worsening is associated with an elevated PAH-AH activity (36, 95% CI: 32-41 vs. 45, 95% CI: 40-51; p=0.04). PAH patients with a mean pulmonary arterial pressure>50 mmHg, and with CRP>4mg·L-1 have increased PAH-AH activity (35, 95% CI: 30-41 vs. 47, 95% CI: 43-52; p=0.02; 37, 95% CI: 32-42 vs. 45, 95% CI: 40-51; p=0.02, respectively). Non-operable CTEPH patients with PAH-AH activity>50 nmol·mL-1 min have a better survival (p=0.02).

Our results suggest that PAH-AH could be a prognostic factor in pulmonary hypertension.

**P3332**

**Hypoxia-mediated alterations in adenosine receptor expression in rat lung**

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**Background:** Chronic hypoxic exposure induces pulmonary arterial remodeling, resulting in pulmonary hypertension (PAH) and right ventricular hypertrophy. The role of adenosine (Ado) receptors in the pathogenesis of PAH has not been addressed.

**Aim and objectives:** To investigate the role of Ado and Ado receptor signaling in hypoxia-induced pulmonary vascular remodeling.

**Methods:** Sprague Dawley rats were exposed to hypobaric hypoxia (5,000 m) for 1 week. The expression profile of the Ado receptors is regulated by hypoxia and hypoxic rat lungs as well as in rat pulmonary artery and microvascular endothelial cells was determined by real time PCR. Ado receptor agonist treated and untreated endothelial cell (EC) proliferation was determined using CyQuant cell proliferation kit.

**Results:** All four Ado receptors were expressed in the lung tissue. The A2A receptor was the most abundant. The 1 week hypoxic exposure significantly upregulated A1 receptor expression indicating its role in the adaptive response to hypoxia. The pulmonary arterial pressure (PAP) were significantly elevated after 3 weeks of hypoxia (33±2.3 versus 18±2.3 normoxia). Studies in vitro revealed that Ado receptors are differentially expressed in pulmonary vascular endothelial cells and that the adenosine receptor A1 (N6-cyclopentadenoine) and A3 (HEMADO) agonists affects EC proliferation.

**Conclusions:** The expression profile of the Ado receptors is regulated by hypoxia and targeting adenosine receptors might be promising approach to treat PAH.

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**P3333**

**Potential contribution of precursor cells to vascular remodeling in the AdTGF-β1 model of lung fibrosis and pulmonary hypertension**

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Pulmonary hypertension (PH) is associated with increased mortality in patients with idiopathic pulmonary fibrosis (IPF). The interaction between the fibrotic process and the pulmonary vasculature is incompletely understood. The current study aimed to investigate whether broad spectrum caspase inhibition can reduce severe angioproliferative PH in the combined model of AdTGF-β1 lung fibrosis and the VEGF receptor inhibitor SU5416.

Female Sprague Dawley rats received AdTGF-β1 intratraehytrally at day 0, as well as one dose of SU5416 s.c. or CMC. Some AdTGF-β1/SU5416 animals received the caspase inhibitor Z-Asp-CH2-DCB or vehicle (DMSO) from day 6-28 At day 28, invasive pulmonary hemodynamics were assessed. The right lung was used for protein and RNA isolation, and the left lung was inflated with formalin and processed for histology.

We detected clusters of VWF+ endothelial cells occluding the lumen of small pulmonary arteries in AdTGF-β1/SU5416 rats with severe PH in AdTGF-β1/SU5416 rats. At the same time, lung fibrosis was increased, as indicated by elevated mRNA expression of profibrotic and matrix genes. Western blot showed a significant increase in caspase-3 cleavage in AdTGF-β1/SU5416 rats. Treatment with Z-Asp-CH2-DCB reduced right ventricular systolic pressures by 19.4 mmHg in average in AdTGF-β1/SU5416 animals (P<0.05 vs. DMSO). In conclusion, our results indicate that angioproliferative pulmonary vasculopathy was induced in this new model, together with severe fibrosis, and that increased apoptosis contributes to both increased fibrosis and vascular pathology.

**P3335**

Effects of type I, II and III interferons on endothelin-1 release by human pulmonary artery smooth muscle cells

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The potent vasconstrictor and mitogen peptide endothelin-1 (ET-1) is a therapeutical target for the treatment of pulmonary hypertension. Work from our group has shown that ET-1 release by human pulmonary artery smooth muscle cells (HPASMCs) is critically regulated by interferons (IFNs) and TNF. We have shown that type I IFNs and IFNγ and type II IFNα, but not type III IFNλ, both released in host responses to viral infection, induce ET-1. As viral infection and IFN therapy are increasingly associated with lung toxicity, including pulmonary hypertension, we have investigated the nature of any interaction between IFNs for ET-1 release by HPASMCs. Cells from 3 separate donors were stimulated in 96-well plates with IFNs, IFNγ, γ- and - and - (all 10ng/ml). Supernatants were collected after 24 hours and ET-1 concentrations measured by sandwich ELISA. In the presence of TNFs (10ng/ml), type I IFNs (a and d) or type II IFNγ, but not type III IFNλ, induced ET-1 release. Additive release of ET-1 was seen with IFNα and IFNγ but not IFNα. IFNs did not release ET-1 under any condition studied. Type I and II IFNs act independently to stimulate ET-1 from HPASMCs, which reflects what is known about their separate receptor pathways.

**Figure 1. Data is mean ± SEM. *P<0.05 one-way ANOVA for combination of IFNs vs IFNs alone.**

Our finding that IFNγ is inactive in these cells may suggest that type III IFN sparing the lung vasculature.

**P3336**

Roles of sex hormones on bone morphogenetic protein signaling in pulmonary artery differed between testosterone and estrogen

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**Background:** Epidemiologic studies have revealed the female predominance in the morbidity of idiopathic PAH (pulmonary arterial hypertension) in the world,
suggesting involvement of sex hormones in the pathogenesis of PAH. Recent studies have identified a role of bone morphogenetic protein (BMP) signaling in the pathogenesis of PAH and we reported that BMP signaling in pulmonary arterial endothelial cells (PAEC) was attenuated under hypoxic conditions in vitro and in vivo.

**Purpose:** The aim is to investigate effects of estradiol (E) and testosterone (T) on the BMP signaling in PAEC and analyze their mechanisms.

**Materials and methods:** PAEC were cultured and incubated with β-estradiol (10^{-7} M), testosterone (10^{-8} M), or vehicle under 1%O₂ (hypoxia) and 21%O₂.

**Results:** Under normoxia, p-Smad1/5/8 protein and Id1 mRNA were upregulated 1.6 and 1.5-fold by E, but suppressed 0.3 and 0.4-fold by T. Under hypoxia, conversely, p-Smads1/5/8 protein and Id1 mRNA were suppressed 0.5 and 0.4-fold by E, but augmented 3.2 and 2.4-fold by T. BMP inhibition led to alteration of BMP signaling similar to hypoxia, whereas HIF-1α inhibitor altered the signaling similar to normoxia.

Discrepancies between hormones could change BMP signaling in PAEC depending on oxygen concentration. Our observations provide the new mechanism how sex hormones affect on BMP signaling, and sex hormones may be novel therapeutic targets in the treatment of PAH.

**P3337** Pulmonary hypertension in the newborn GTP-cyclohydrolase 1 deficient mouse is unrelated to endothelium-dependent vasorelaxation potential

**Background:** Leptin and its receptor are increased in PAH patients, whereas Treg function is inhibited. Interestingly, IPAH and CTD-PAH are comparable. Therefore, leptin and its receptor could play an important role in the immunopathogenesis of PAH. Support: Medical Research Foundation Josso Award 2010.

**P3339** Risk factors for elevated liver function tests (eLFTs) in patients with pulmonary arterial hypertension (PAH) treated with sitaxentan and followed in an European safety register

**Background:** Endothelin receptor antagonists, including sitaxentan (Thelin®), treat PAH but may cause hepatotoxicity. The outcomes for Patient Surveillance (TOPS) safety registry monitored prospectively events in European patients (pts).

**Aim:** Describe risk factors for eLFTs in TOPS pts.

**Methods:** Sites entered data for eligible pts monthly from baseline to discontinuation; eLFTs were defined as ALT or AST levels >3xULN. Logistic regression assessed effects on subsequent eLFTs of: treatment exposure (mo), age (by 10-yr periods), functional class (NYHA IV vs I/II), etiology (idiopathic vs associated), presence or absence of each variable: female sex; Caucasian race; prior PAH therapy failure; previous bosentan or ambrisentan; concomitant sildenafil or tadalafil; baseline LFT elevation.

**Results:** Of 932 pts, ALT or AST was >3xULN in 97.6% (1.1% missing) at baseline and 83.7% (11.5% missing). The eLFTs at 4.9% (4.6%) during followup were lower (54 vs 61 Yr), had shorter treatment (9 vs 14 mo), and had higher baseline ALT/LN and AST/LN ratios vs pts without eLFTs during followup (all P<0.01). Age (odds ratio [OR], 0.74; 95% CI, 0.60–0.91), female sex (OR, 1.5; 95% CI, 0.97–2.3) and baseline LFT elevation (OR, 9.4; 95% CI, 1.5–60.3) were associated with eLFTs at followup (all P<0.02).

**Conclusions:** 4.9% of pts receiving sitaxentan had eLFTs during followup; age, treatment exposure, and baseline eLFTs were significantly associated with eLFTs during followup.

**P3340** Increased p38α activity induces smooth muscle cell proliferation and migration in idiopathic pulmonary arterial hypertension.

**Background:** The role of p130Cas in iPAH and control PAH is currently unknown.

**Methods:** Protein and phosphorylation levels of p38α were quantitated by Western blot in surgically resected lung specimens from iPAH-patients and normal lung specimens.

**Results:** The p38α protein was increased 1.6 and 1.5-fold by E, but suppressed 0.3 and 0.4-fold by T. Under hypoxia, protein and phosphorylation were suppressed 0.5 and 0.4-fold by E, but augmented 3.2 and 2.4-fold by T. BMP inhibition led to alteration of BMP signaling similar to hypoxia, whereas HIF-1α inhibitor altered the signaling similar to normoxia.

**Conclusion:** Leptin and its receptor could play an important role in the immunopathogenesis of PAH. Support: Medical Research Foundation Josso Award 2010.
Objective: This study aims to investigate the interaction between ghrelin and local RAS from rat pulmonary vessels during ovulobolin – induced allergic airway disease.

Methods: The angiotensinogen (AGT) – induced contractions were assessed on isolated pulmonary artery and veins from ovulobolin sensitized rats receiving either saline (OSS) or ghrelin (OSG) by endothelial intact. Experiments were performed in the absence or the presence of lisuratin, D-ALA7, chymostatin, and N-nitro-L-arginine methyl ester (L-NAME).

Results: The angiotensinogen (AGT) contractile effects mediated by AT1 receptors were shown with at least 25% on vessels from OSG compared to from OSS. The D-ALA7 and LNAME significantly increases the AGT - induced contraction on OSS. The amount of nitric oxide released after stimulation with angiotensinogen (AGT) is higher on OSG and it is blocked by D-ALA7.

Conclusion: Our results suggested that pulmonary delivery of ghrelin could modulate the local RAS from pulmonary vessels, probably by promoting the angiotensin 1-7 mediated effects. These data sustained the existence of another possible way for ghrelin’s beneficial effects on the lung.

P3342 Nestin expressing progenitor cells in pulmonary vasculature

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Vascular smooth muscle cells (VSMCs) and pericytes (PCs), distinguished by the expression of neuronal stem cell marker “Nestin”, may represent stem cell-like progenitor cells for tissues in various organs. In one of our previous studies, we found that PCs in stentless blood vessels are the progenitors of testosterone producing Leydig cells.

To analyze the expression pattern of nestin and its role as marker for proliferating progenitor cells in the lung, nestin expression and localization was investigated during postnatal development in nestin-GFP mice. To investigate nestin expression during vascular remodelling, samples from two models of pulmonary hypertension (PH) [monocrotaline (MCT) rat model and hypoxic mouse model] as well as human samples from patients of PH were analyzed. Nestin data was compared with expression of proliferation markers (PCNA, Ki67) and PDGF receptors.

Nestin was found in a subpopulation of VSMCs and PCs of lung vasculature. As compared to normoxic controls significantly higher nestin expression was observed in pulmonary vasculature of postnatal tissues and in adult lungs between day 3-7 of hypoxic exposure but not at later time points when PH became evident. Increase of nestin correlated well with an increase of cell proliferation. In hypoxic lungs peak of phosphorylated (activated) PDGF receptor β correlated with nestin one. Increase of nestin-immunoreactive VSMCs and PCs was also found in MCT rat and human lung samples.

Certain contractile cells capable of proliferation could be identified by Nestin expression in lungs and may be used as prognostic marker and new target for therapeutic interventions of diseases like PH.

P3343 Endothelial cell mechanics are altered in pulmonary arterial hypertension (PAH)

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Idiopathic pulmonary arterial hypertension (IPAH) and collagen vascular disease associated PAH (APAH) are associated with a significant elevation of inflammasome activation and release of IL-1β and IL-6 in patient plasma [1]. We hypothesized that these biochemical changes will affect intercellular force distribution in the constituent endothelial cells. To test this hypothesis, we were applied to cultured Human Lung Microvascular Endothelial Cell (HMVEC), serum derived from IPAH and APAH patients, and measured subsequent changes in HMVEC intercellular forces [2]. In comparison to time-matched controls (n=4), HMVEC monolayers exposed to patient serum (n=5, APAH; n=8) were significantly more contractile (average contractile moment per monolayer; control cells = 153.5 + 13.5 pNm, IPAH cells = 222.3 + 9.6 pNm, and APAH cells = 223.8 + 22.8 pNm) and exhibited greater number of intercellular stress hot-spots. Accordingly, we suggest that inflammasome mediated enhancements in endothelial intercellular forces may play an important role in decreased vascular compliance observed in PAH.

References:
have also observed perturbed expression of TGFBR3 in the lungs of neonatal mice with hyperoxia (85% O₂)-induced lung injury, which results in bronchopulmonary dysplasia (BPD). The mRNA levels (assessed by quantitative real-time RT-PCR) for \( \text{tgfbr3} \) were downregulated (4.4-fold, p=0.003), while TGFBR3 protein levels were downregulated by 70%. Laser capture microdissection confirmed dysregulated expression of TGFBR3 in the pulmonary vasculature of the developing mouse lung. Taken together, these data suggest a role for TGFBR3 in vascular smooth muscle cell function which could lead to a dysregulation of TGF-β signalling in the pulmonary vasculature, which in turn could contribute to the impaired pulmonary vascular growth and development associated with the lung hypoplasia observed in patients with BPD.

**P3347**

**Elevated levels of adenosine in the lungs lead to chronic lung injury and pulmonary hypertension**

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Pulmonary Hypertension (PH) is characterized by increased pulmonary vascular tone and remodeling of the pulmonary vasculature including muscularization of vessels. PH is often associated with underlying chronic lung diseases (CLD) such as chronic obstructive pulmonary disease (COPD) and idiopathic pulmonary fibrosis (IPF). The adenosine (Ado) A2B receptor (R) expression is increased in patients with COPD and IPF. Activation of the A2BR by Ado has been shown to regulate fibrosis through its action in inflammatory and structural cells. However, the role of Ado and the A2BR in the pathogenesis of PH is not known.

**Hypothesis:** Ado acting on the A2BR modulates the development of PH in CLD. Ado deaminase (ADA)-deficient mice have increased levels of Ado in the lung tissue that lead to CLD. On day 30, once lung injury was established, mice were provided with chow containing placebo or GS-6201, an A2BR antagonist, for the next 10 days. On day 41, right ventricle systolic pressure (RVSP), systemic blood pressure, heart rate and lung function measurements were performed. ADA-deficient mice had increased RVSP compared to control mice. Lung function measurements revealed increased airway resistance and a reduction in airway and tissue compliance in ADA-/- mice. Blockade of the A2BR by GS-6201 inhibited the increased RVSP and restored lung function. No change in systemic systolic blood pressure or heart rate was observed in mice treated with placebo or GS-6201.

These results highlight the role of the A2BR in the pathogenesis of PH associated with elevated tissue Ado and CLD. The results suggest that targeting the A2BR could be a potential target for the treatment of PH secondary to CLD.