Pulmonary arterial hypertension: scientific advances

**S34** BMPR-II DEFICIENCY LEADS TO AN INCREASE IN LUNG EGG DEPOSITION, PULMONARY VASCULAR REMODELLING AND AN ABNORMAL LIVER VASCULATURE IN MICE CHRONICALLY INFECTED WITH S. MANSONI

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**Rationale and objectives** Schistosomiasis is the world-wide leading cause of pulmonary arterial hypertension (PAH) and is particularly prevalent in developing countries. More than 80% of patients with familial PAH in the western-world have a mutation in bone morphogenetic protein type-II receptor (BMPR-II), which is a member of the transforming growth receptor-beta (TGF-b) superfamily and is important in cell proliferation and differentiation. The aim of the study was to determine if mice with a heterozygous null mutation in BMPR-II are more susceptible to pulmonary vascular remodelling induced by *S. mansoni* infection, compared with wild-type littermates.

**Methods** Wild-type (BMPR-II+/+) and BMPR-II heterozygous (BMPR-II +/-) C57/BL6 mice were infected percutaneously with *S. mansoni*. Seventeen weeks post-infection right ventricular systolic pressure (RVSP), right ventricular hypertrophy (RVH), liver and lung egg counts were measured. Pulmonary vascular remodelling and liver histology were assessed by morphometry, following immunohistochemistry. Lung, liver and serum cytokines were also measured. A macrophage phagocytosis assay and in vivo bead assay were also performed.

**Measurements and main results** At 17 weeks post-infection there was a significant increase in pulmonary vascular remodelling associated with a significant increase in egg deposition and cytokines in the lung, in BMPR-II+/+ mice. Furthermore, there was a positive correlation between lung egg deposition and pulmonary vascular wall thickness. Additionally, there was a significant dilatation of the central hepatic vein in the BMPR-II+-/-infected mice compared with the BMPR-II+/+ infected mice. However, no differences in RVSP, RVH or liver egg deposition were found.

**Conclusions** This study has shown that mice deficient in BMPR-II are more susceptible to pulmonary vascular remodelling induced by *S. mansoni* which is directly correlated to an increase in egg burden in these mice. Additionally, we have shown that BMPR-II+/- mice have an abnormal liver vasculature, which may be responsible for increased egg shunting into the lungs.

**S35** BMPS AND BMP10 MEDIATE CONNECTINX EXPRESSION IN ENDOTHELIAL CELLS: IMPLICATIONS FOR PAH AND HHT


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**Background** Germ-line mutations in the bone morphogenetic protein type-II receptor, BMPR-II, underlie 80% of heritable...
pulmonary arterial hypertension (PAH) cases and approximately 25% of idiopathic PAH cases. PAH may arise due to endothelial dysfunction as mice with BMPR-II deficiency exhibit increased pulmonary vascular permeability.

BMP9 is an endothelial quiescence factor and is thought to maintain the integrity of the endothelium. We previously reported that BMPR-II and ALK1 are the key receptors through which BMP9 inhibits the proliferation of human pulmonary artery endothelial cells (hPAECs). We hypothesised that BMPR-II deficiency impacts on endothelial cell connectivity and may contribute to endothelial dysfunction in PAH.

Methods Human pulmonary artery endothelial cells were obtained from Lonza and blood outgrowth endothelial cells (BOECs) were isolated from peripheral blood of unaffected controls or PAH patients with identified BMPR-II mutations. Cells were transfected with siRNAs targeting BMPR-II followed by stimulation with BMP9. RNA was extracted and the expression of candidate genes determined by quantitative PCR. Further siRNA studies were performed for ALK1 and endoglin siRNAs. The promotion of gap junction assembly by BMP9 and BMP10 were assessed by immunofluorescence, Western blotting and functionally using Parachute assays.

Results Screening of candidate BMP9-induced junctional and structural proteins highlighted a subset of endothelial connexins that are BMP9 and BMP10-responsive and dependent on BMPR-II and ALK1. BMP9 and BMP10 increased the expression of the connexins, assessed by Western blotting and immunostaining. In addition, BMP9 and BMP10 significantly increased the transfer of calcine from labelled donor cells to unlabelled acceptor cells, indicating a promotion of endothelial cell connectivity.

Conclusion In addition to their roles promoting endothelial quiescence, BMP9 and BMP10 directly promote endothelial cell connectivity by increasing connexin expression and assembly. The central contributions of BMPR-II and ALK1 to this process may implicate impaired endothelial cell connectivity as a pathological component of PAH and HHT.

S36 FERROPORTIN IS EXPRESSED IN HUMAN PULMONARY ARTERY SMOOTH MUSCLE CELLS: IMPLICATIONS FOR PULMONARY ARTERIAL HYPERTENSION

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Background Pulmonary Arterial Hypertension (PAH) is a rare but fatal condition manifested by pulmonary vascular remodelling, increased pulmonary vascular resistance and right-heart failure. Disruption in iron handling and anaemia, caused by elevated iron-regulatory hormone hepcidin, is observed in PAH. Ferroportin is the only known cellular iron-export protein that is downregulated by hepcidin. As such, iron supplementation as a therapy is currently under clinical trial. However, it is also known that iron is both pro-oxidant and pro-proliferative. Latest evidence also points to sub-clinical haemolysis and the presence of free haemoglobin in PAH patients. We hypothesised that ferroportin would be expressed; be responsive to hepcidin challenge and have implications for the proliferation of human pulmonary artery smooth muscle cells (hPASMCs).

Methods The mRNA levels of ferroportin was measured by RT-PCR, the protein expression was detected by western-blot analysis and quantified by ELISA. The sub-cellular distribution of ferroportin was visualised by immunocytochemistry (ICC). hPASMCs were pre-incubated with or without free haemoglobin and further challenged with increasing doses of hepcidin and the proliferative responses assessed by cyquant and/or BrdU incorporation assays. Some cells were also pre-incubated with LY2928057 (monoclonal antibody against ferroportin that stabilises cellular expression, Eli-Lilly) in proliferation assays.

Results Basal ferroportin mRNA was detected in hPASMCs, but the mRNA levels were largely unaltered with hepcidin exposure (n = 3). A ~50KDa protein band representing ferroportin was detected under resting conditions while hepcidin challenge caused decrease in ferroportin protein levels (Figure 1). Basal ferroportin was uniformly distributed in the cells; however hepcidin treatment led to intense punctate/vesicular staining (n = 3). Finally, exposure to free haemoglobin alone or along with hepcidin increased proliferation of hPASMCs by 13.6% and 12.4% (p < 0.05, n = 3) respectively. Interestingly, pre-incubation of the cells with LY2928057 partly reversed this effect.

Conclusion This is the first report of ferroportin expression and regulation in hPASMCs. We suggest that targeting and manipulating the hepcidin-ferroportin axis using LY2928057 might prove a novel therapeutic approach for PAH.

S37 VASCULAR ENDOTHELIAL CELL GROWTH FACTOR-A (VEGF-A) SIGNALLING AND NEOVASCULARISATION OF PULMONARY ENDARTERECTOMY MATERIAL IN CHRONIC THROMBOEMBOLIC PULMONARY HYPERTENSION (CTEPH)

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Background Despite recent advances in the medical treatment of patients with CTEPH, relatively little is understood surrounding the underlying pathological mechanisms. Many patients have a historical documented venous thromboembolic event (VTE) and consequently, failed resolution of an acute VTE has been proposed as a key initiating factor in the subsequent development of CTEPH. Here we investigated VEGF-A levels, a key regulator of angiogenesis, in CTEPH patients prior to and following