Cell signalling and cell responses in pulmonary vascular disease

S150 SMAD-DEPENDENT AND SMAD-INDEPENDENT INDUCTION OF ID1 BY PROSTACYCLIN ANALOGUES INHIBITS PROLIFERATION OF PULMONARY ARTERY SMOOTH MUSCLE CELLS IN VITRO AND IN VIVO

doi:10.1136/thx.2010.150953.1

Jun Yang, Xiaohui Li, Rafia S Al-Lamki, Mark Southwood, Jing Zhao, Andrew M Lever, Friedrich Grimminger, Ralph T Schermuly, Nicholas W Morrell. *University of Cambridge, Cambridge, UK*

Introduction and Objectives Mutations in the bone morphogenetic protein type II receptor (BMPR-II) are responsible for the majority of cases of heritable pulmonary arterial hypertension (PAH). Mutations lead to reduced Smad1/5-driven expression of inhibitor of DNA binding protein 1 (Id1) and loss of the growth suppressive effects of BMPs. The impact of existing PAH therapies on BMP signalling is lacking. Because prostacyclin analogues are effective treatments for clinical PAH, we hypothesised that these agents enhance Smad1/Id1 signalling.

Methods Iloprost alone induced Id1 expression in human pulmonary artery smooth muscle cells (PASMCs), an effect that was independent of Smad1/5 activation but dependent on a cAMP-responsive element in the Id1 promoter. In addition, iloprost and treprostinil enhanced BMP-induced phosphorylation of Smad1/5 and Id1 expression in a cAMP-dependent manner. The mechanism involved suppression of inhibitory Smad, Smad6. Furthermore, iloprost rescued the deficit in Smad1/5 phosphorylation and Id gene expression in PASMCs harbouring mutations in BMPR-II and restored growth suppression to BMP4 in mutant PASMCs.

Results We confirmed a critical role for Id1 in PASMC proliferation. Reduced expression of Id1 was observed in concentric intimal lesions of heritable PAH cases. In the monocrotaline rat model of PAH, associated with reduced BMPR-II expression, we confirmed that treprostinil inhibited smooth muscle cell proliferation and prevented progression of PAH while enhancing Smad1/5 phosphorylation and Id1 gene expression.

Conclusions Prostacyclin analogues enhance Id1 expression in vitro and in vivo and restore deficient BMP signalling in BMPR-II mutant PASMCs.

S151 TRAIL DEFICIENCY IS PROTECTIVE IN EXPERIMENTAL PULMONARY ARTERIAL HYPERTENSION

doi:10.1136/thx.2010.150953.2

[1]A G Hameed, [1]J Chamberlain, [1]N D Arnold, [1]S E Francis, [1]C M H Newman, [2]D C Crossman, [1]A Lawrie. *[1]University of Sheffield, Sheffield, UK; [2]NIHR Cardiovascular Biomedical Research Unit, Sheffield, UK*

Introduction and objectives Despite advances in the overall management of Pulmonary Arterial Hypertension (PAH) significant morbidity and poor prognosis remain a major clinical problem. Identifying key pathways in the pathogenesis of this disease will allow development of more targeted therapies aimed at treating PAH. There is emerging evidence to support that Tumour Necrosis Factor (TNF)-Related Apoptosis-Inducing Ligand (TRAIL) plays an important role in vascular biology. We have recently reported expression of TRAIL from lesions of patients with advanced Idiopathic PAH. To further determine the role of TRAIL in the pathogenesis of PAH we used a diet-induced murine model of PAH.

Methods ApoE$^{-/-}$, TRAIL$^{-/-}$ and ApoE$^{-/-}$/TRAIL$^{-/-}$ double null mice were fed chow or Paigen (high fat, cholate-containing diet) for 8 weeks. They underwent echocardiographic assessment prior to right heart catheterisation using the internal jugular venous route. The heart and right lung were perfusion fixed with 10% formalin for subsequent determination of right ventricular mass and Immunohistochemistry of pulmonary vascular lesions. The left lung was immediately frozen in liquid nitrogen for subsequent determination of protein and RNA by western Immunoblotting and quantitative PCR. Identical to above ApoE$^{-/-}$/TRAIL$^{-/-}$ double null mice were also treated with recombinant murine TRAIL, or saline (4 week infusion via an osmotic mini pump) at the time of commencing the diet for 8 weeks.

Results Compared to control chow fed mice, ApoE$^{-/-}$ mice fed the Paigen diet developed significant elevation of Right Ventricular Systolic Pressure (RVSP) (23 mm Hg vs 50 mm Hg n=7 $p<0.001$), pulmonary vascular resistance and arteriolar remodelling. ApoE$^{-/-}$/TRAIL$^{-/-}$ double null mice fed the Paigen diet, were protected from these haemodynamic (RVSP 27 mm Hg n=6 $p<0.05$) and pulmonary vascular remodelling changes. Moreover, the PAH phenotype was re-established in the ApoE$^{-/-}$/TRAIL$^{-/-}$ double null mice by the administration of exogenous recombinant TRAIL.

Conclusions TRAIL is a critical mediator in disease pathogenesis of PAH in the diet-induced murine model of PAH. Targeting TRAIL could provide a novel therapeutic approach to the treatment of PAH. Work is ongoing to determine if this approach can stabilise or reverse established disease, in both this, and rat, experimental models of PAH.

S152 DEXAMETHASONE REVERSES ESTABLISHED MONOCROTALINE-INDUCED PULMONARY HYPERTENSION IN RATS AND INCREASES PULMONARY BMPR2 EXPRESSION

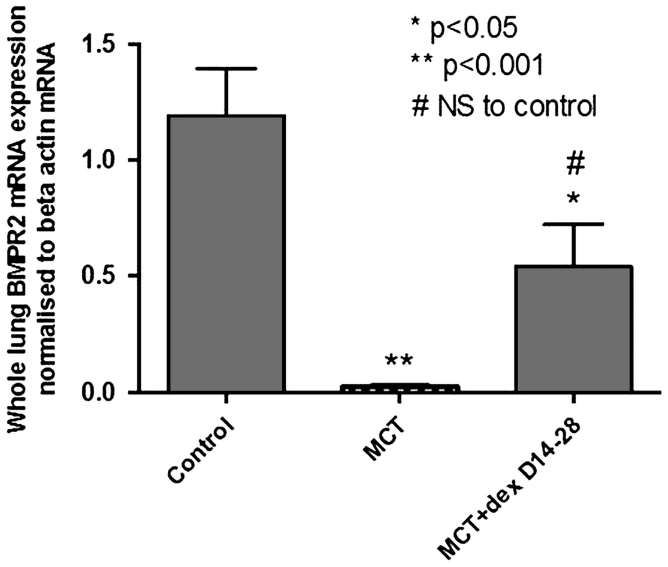
doi:10.1136/thx.2010.150953.3

[1]L C Price, [1]S J Wort, [2]D Montani, [2]C Tcherakian, [2]P Dorfmuller, [2]R Souza, [1]D Shao, [2]G Simonneau, [3]L S Howard, [1]I Adcock, [2]M Humbert, [2]F Perros. *[1]National Heart & Lung Institute, Royal Brompton Hospital, London, UK; [2]Centre National de Référence de l'HypertensionPulmonaireSévère, Hôpital Antoine Béclère, Clamart, F-92140, France; Université Paris-Sud, Paris, France; [3]Hammersmith Hospital, Imperial College Healthcare NHS Trust, Du Cane Road, London, UK*

Background Pulmonary arterial hypertension (PAH) is associated with pulmonary vascular inflammation and dysregulated bone morphogenetic protein receptor type 2 (BMPR2) signalling in both human and experimental PAH. We evaluated the effects of dexamethasone on established monocrotaline-induced PAH in rats for potential reversal of PAH, at time points when pulmonary vascular remodelling has already developed (from day 14 after a single injection of monocrotaline at day 0), and for the effects on pulmonary IL6 and BMPR2 expression.

Methods Saline-treated controls, monocrotaline-exposed, monocrotaline-exposed and dexamethasone-treated rats (5 mg/kg/day, 1.25 mg/kg and 2.5 mg/kg/48 h given from day 14–28 and day 21–35) were evaluated at day 28 and day 35 following monocrotaline for pulmonary vascular haemodynamic parameters, right ventricular hypertrophy, morphometry, immunohistochemistry, whole lung *IL-6* and *BMPR2* expression by quantitative real-time PCR (qRT-PCR).

Results Dexamethasone significantly improved pulmonary haemodynamics and morphometric indices of pulmonary vascular remodelling, reversing PAH when given at day 14–28, day 21–35 following monocrotaline, as well as improving survival in monocrotaline-exposed rats compared to controls (log rank $p<0.0001$). Dexamethasone reduced both monocrotaline-induced whole lung *IL-6* overexpression ($p<0.05$), as well as reducing IL-6-expressing adventitial inflammatory cell infiltration as assessed by immunohistochemistry. This was associated with pulmonary *BMPR2*



Abstract S152 Figure 1 Effects of monocrotaline and day 14–28 dexamethasone on whole-lung BMPR2 expression by qRT-PCR.

down-regulation ($p<0.01$) following monocrotaline, which was significantly increased following day 14–28 dexamethasone treatment in whole lung ($p<0.05$) (Abstract S152 Figure 1). Cellular *BMPR2* was also increased following *in vitro* treatment of control pulmonary artery smooth muscle cells (PASMC) with $\times 10^{-8}$ molar dexamethasone ($p<0.05$), but not in PASMC isolated from pulmonary hypertensive rats. Dexamethasone ($\times 10^{-8}$ and 10^{-7} molar) also reduced proliferation of PASMC isolated from both control and pulmonary hypertensive rats ($p<0.05$ for both doses).

Conclusion PAH in this well-characterised experimental model can be reversed by dexamethasone, and survival is improved. In this model, mechanisms may involve reduction of IL-6-expressing inflammatory cells, reduced proliferation of vascular smooth muscle cells, and restoration of pulmonary *BMPR2* expression may be important.

S153 BONE MORPHOGENETIC PROTEIN RECEPTOR-II REGULATES PULMONARY ARTERY ENDOTHELIAL CELL BARRIER FUNCTION: RELEVANCE TO HERITABLE PULMONARY ARTERIAL HYPERTENSION

doi:10.1136/thx.2010.150953.4

[1]V J Burton, [1]L I Ciuclan, [1]A M Holmes, [2]D Rodman, [1]C Walker, [1]D C Budd. *[1]Respiratory Disease Area, Novartis Institutes for BioMedical Research, Wimblehurst Road, Horsham, West Sussex RH12 5AB, UK; [2]Respiratory Translational Medicine, Novartis Institutes for BioMedical Research 220 Massachusetts Avenue, 3rd floor, Cambridge, MA 02139, USA*

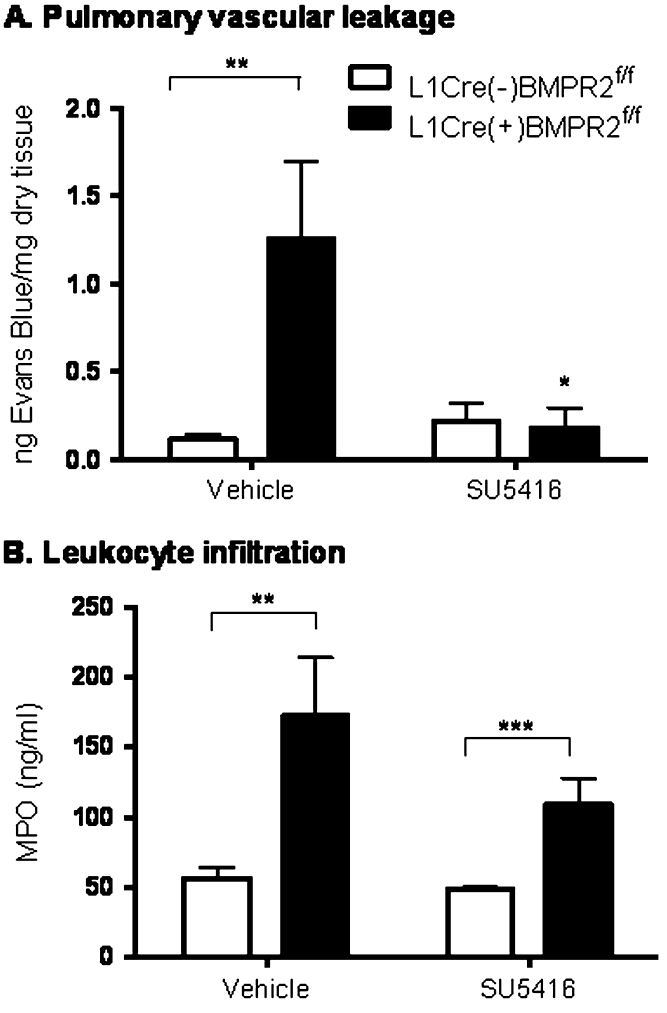
Background Mutations in bone morphogenetic protein receptor II (BMPR-II) have been shown to underlie most heritable cases of Pulmonary arterial hypertension (PAH). However, less than half the individuals who harbour mutations develop the disease. This fact has lead to speculation that the genetic defect combined with an additional trigger, such as inflammation, may be required for the disease to be manifested.

Aim To define the role of BMPR-II in regulating the barrier function of pulmonary artery endothelial cells (PAEC).

Methods In vitro, BMPR-II expression was reduced in HPAEC using siRNA and cells were seeded onto transwell filters. FITC-labelled albumin (to assess permeability) or leukocytes (to assess leukocyte migration) were added to the upper chamber and either leakage of FITC-albumin or transmigration of leukocytes into the lower chamber assessed over time. Using a flow-based model, TNFα (4 h) or TGFβ1 (24 h) stimulated HPAEC were seeded into Ibidi slides and leukocyte-endothelial interactions visualised, recorded and quantified. In vivo, permeability was assessed by measuring Evans blue leakage into the pulmonary vasculature in endothelial restricted BMPR-II deficient mice. Myeloperoxidase (MPO) in the lungs was assessed as a measure of leukocyte infiltration.

Results Leakage of FITC-albumin through HPAEC with reduced BMPR-II expression was significantly increased compared to mock-transfected HPAEC. Under static and flow conditions, leukocyte transmigration was greatly increased through HPAEC with reduced BMPR-II expression following TNFα or TGFβ1 stimulation. This facilitated transmigration following loss of BMPR-II could be blocked by pharmacological intervention of CXCR2.

Conclusions Our data suggest a novel role for BMPR-II in dampening inflammatory signals in the pulmonary vasculature and that loss of BMPR-II in the endothelial layer of the pulmonary vasculature may lead to heightened susceptibility to inflammation-induced tissue damage. We speculate this may be a key mechanism involved in the initiation of the disease in heritable PAH that result from defects in BMPR-II expression.



Abstract S153 Figure 1