Mutations in BMPR2 are the major cause of familial pulmonary arterial hypertension (PAH). Reduced BMPR-II expression is significantly reduced in both familial and idiopathic PAH patients. We have shown previously that BMPR-II expression is regulated via a lysosomal degradative pathway. The anti-malarial drug, chloroquine, blocks lysosomal degradation by raising lysosomal pH and impairs autophagic protein degradation. Using an experimental rat model of PAH we observed that chloroquine administration prevented an increase in right ventricular systolic pressure (RVSP), right ventricular hypertrophy (RVH) and vascular remodelling following monocrotaline (MCT) treatment. BMPR-II expression was significantly increased in lungs from chloroquine treated rats. Furthermore, in cellular localisation studies chloroquine increased BMPR-II cell surface expression.

Methods Male Sprague-Dawley rats received a single subcutaneous injection of MCT to induce PAH. To assess prevention or inhibition of PAH progression, animals received chloroquine, or vehicle, by daily intraperitoneal injection from day 1 to 21 or day 21 to 51 post MCT injections, respectively. Rats were anaesthetised for haemodynamic assessment and lung tissue collected for immunohistochemistry and protein isolation. Muscle and lung tissue was assessed in frozen tissue sections by staining with anti-smooth muscle actin. BMPR-II protein expression was determined in frozen lung tissue using western blotting. Cellular localisation of BMPR-II expression in H1 fibroblast cell line that stably expresses green fluorescent protein (GFP) tagged BMPR-II was determined using immunofluorescence and biotinylation of cell surface BMPR-II using NHS-Biotin-SS labelling and precipitation with avidin agarose beads. BMPR-II cell surface expression was then determined by GFP immunoblotting.

Results Chloroquine prevented experimental PAH by significantly decreasing RVSP, RVH and muscle/blood ratio in MCT-treated rats. Treatment with chloroquine dramatically increased BMPR-II protein levels in the lung. Furthermore, chloroquine treatment inhibited MCT-induced PAH. This was associated with an increase in cell surface BMPR-II expression in a cell line that stably expresses the GFP-tagged receptor.

Conclusion This study demonstrates the potential use of chloroquine as a therapeutic agent in the treatment of PAH by potentially increasing levels of BMPR-II at the cell surface.

S101 HETEROZYGOUS LOSS OF BMPR-II PREDISPOSES TO INFLAMMATORY CYTOKINE SECRETION AND PULMONARY VASCULAR SMOOTH MUSCLE PROLIFERATION

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Introduction Elevated levels of inflammatory cytokines are associated with idiopathic and heritable pulmonary hypertension (PAH) and predict mortality, but their role in the pathobiology of PAH and the underlying mechanisms remain unclear. We investigated whether loss of bone morphogenetic protein receptor type II (BMPR-II), the underlying cause of heritable PAH, predisposes to increased inflammatory cytokine expression and pulmonary vascular remodelling.

Methods Regulation of cytokine expression by lipopolysaccharide (LPS) was studied in two in vitro models: (1) mouse pulmonary artery smooth muscle cells (PASMCs) heterozygous for a null allele in bmpr2 (BMPR2mut−/+), and (2) human PASMCs with a mutation in BMPR2 (BMPR2mut−) and their wild-type counterparts. Cytokine production, regulation and effects on the pulmonary vascular system were examined in an in vivo model using the bmpr2−/− mouse.

Results Both mouse and human in vitro models showed that loss of BMPR2 function leads to increased mRNA expression and secretion of interleukin 6 and IL-8 both at baseline and after stimulation with LPS. This was associated with loss of expression of antioxidant enzymes such as superoxide dismutase 1 (SOD1) and SOD3, which is demonstrable in both mouse bmpr2−/− and human BMPR2mut− cells. Treatment with the superoxide dismutase-mimetic, Tempol, partially reversed the exaggerated cytokine response to LPS but did not affect the underlying baseline increase. We demonstrated increased phospho-Stat3 signalling and pro-proliferative and pro-survival effects of IL-6 in BMPR2mut− PASMCs which is not seen in the wild-type. This was confirmed in the in vivo model, as bmpr2−/− mice demonstrated increased IL-6 and IL-2 expression in sera, lung and liver tissue 3 and 24 h after exposure to 10 μg of LPS. A similar pattern of antioxidant enzyme reduction was also seen in lung tissue from these LPS-treated bmpr2−/− mice.

S100 THE BONE MORPHOGENETIC PROTEIN TYPE II RECEPTOR IS CRITICAL FOR VENOUS ANGIOSIS IN ZEBRAFISH

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Introduction and Objectives Pulmonary arterial hypertension (PAH) is a rare but severe condition, often fatal within 3-5 years due to right heart failure. Mutations in the bone morpho-genetic protein (BMP) type II receptor (BMPR-II) underlie heritable forms of the disease but the mechanisms leading to vascular disease remain obscure from studies in mice and humans. Here we use zebrafish, which have a well-documented pattern of angiogenesis, as a model organism to address this question.

Methods The transgenic Tg(fli1a:egfp) zebrafish line, where the fli1a promoter drives GFP expression in vascular endothelial and blood cells, was used throughout this work. A variety of methods were used to dissect the role of BMP signalling in vascular development including: (i) BMP receptor inhibitors (dorsomorphin and LDN193189), (ii) antisense morpholino oligonucleotides (morpholinos) and (iii) transgenic zebrafish engineered with heat shock inducible dominant-negative BMP receptors. To identify BMP responsive transcripts from vascular endothelial cells in vivo we developed a system to allow FACS isolation of GFP+ve cells disassociated from Tg(fli1a:egfp) zebrafish following incubation in LDN193189 or DMSO solvent control. The mRNA transcripts in GFP+ve cells were determined using massively parallel (Illumina) sequencing, mapped to the Zv8 zebrafish genome and differences in transcript abundance between LDN193189 and DMSO treated embryos were determined using cufflinks.

Results Inhibition of BMP signalling with LDN193189 in zebrafish embryos after dorso-ventral patterning has occurred blocked venous but not arterial angiogenesis. This phenotype was reproduced in BMPR2 dominant-negative zebrafish and following knockdown of the zebrafish homologues of BMPR2, bmp2a and bmp2b with morpholinos. Illumina sequencing identified the BMP responsive genes in vascular endothelial cells and the gene(s) responsible for the venous angiogenic phenotype are being determined by knockdown of these genes with morpholinos.

Discussion BMP signalling, via BMPR2, is critical for venous but not arterial angiogenesis in zebrafish. BMPR2 mutations are recognised as causative in patients with heritable PAH, where venous involvement is recognised, but also in pulmonary veno-occlusive disease. Our findings provide novel insights onto the potential role of the pulmonary venous system in pulmonary hypertension and implicate abnormal venous angiogenesis as a novel mechanism underlying this disease.