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Pulmonary hypertension: advances in pathogenesis and treatment

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Pulmonary hypertension is an orphan disease that until recently has received limited attention within the wider medical community. This has changed distinctly in the last 10 years with the advent of new classes of therapy and a renewed interest in mechanisms of pathogenesis. This review utilized information gathered from recent conferences, and a review of the literature was conducted using MedLine and Pubmed. Accepted mechanisms of pathogenesis and currently available treatments are presented. We will discuss interesting new concepts in pathogenesis, including the importance of genetic forms of the disease and in particular the transforming growth factor receptor superfamily and the evolving evidence of the contribution of dysregulated immunity. Areas of research may yield therapeutic benefits in the not-too-distant future, including anti-proliferative therapies and stem cell therapy.

Keywords: pulmonary hypertension/vascular remodelling

Established pathophysiology of pulmonary hypertension

Pulmonary hypertension can result from a wide variety of aetiologies. The most recent classification as listed in Table 1 demonstrates that this is not a single disease with a simple aetiology. In this review we will focus largely on idiopathic and heritable disease for the practical reason that this area is where the research in both pathogenesis and treatment has been most clearly established, although in animals hypoxic and inflammatory models have predominated. Despite this the resulting pathophysiology across this wide spectrum of diseases is remarkably similar. Whether this similarity reflects a stereotypic response to increased pressure, or points to more fundamental mechanisms of how the vasculature reacts to different insults, is not yet clear. It is thought that endothelial dysfunction is a primary factor in disease initiation. Endothelial dysfunction leads to an imbalance in the production of vasoconstrictors versus vasodilators, factors affecting smooth muscle cells, thrombotic mediators and inflammatory cytokines.[1] Consequently, there is migration and proliferation of smooth

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Table 1 Clinical classification of pulmonary hypertension.

2009 Dana point classification	
1	PAH: idiopathic PAH, heritable, BMPR2, ALK1, endoglin (with or without hereditary haemorrhagic telangiectasia), unknown, drug- and toxin induced
2	Associated with connective tissue diseases: HIV infection, portal hypertension, congenital heart diseases, schistosomiasis, chronic hemolytic anaemia, persistent pulmonary hypertension of the newborn
3	Pulmonary veno-occlusive disease and/or pulmonary capillary hemangiomatosis
4	Pulmonary hypertension owing to left heart disease: systolic dysfunction, diastolic dysfunction, valvular disease
5	Pulmonary hypertension owing to lung diseases and/or hypoxia: chronic obstructive pulmonary disease, interstitial lung disease, other pulmonary diseases with mixed restrictive and obstructive pattern, sleep-disordered breathing, alveolar hypoventilation disorders, chronic exposure to high altitude, developmental abnormalities
6	Chronic thromboembolic pulmonary hypertension
7	Pulmonary hypertension with unclear multifactorial mechanisms
	Hematologic disorders: myeloproliferative disorders, splenectomy
	Systemic disorders: sarcoidosis, pulmonary Langerhans cell histiocytosis: lymphangioleiomyomatosis, neurofibromatosis, vasculitis
	Metabolic disorders: glycogen storage disease, Gaucher disease, thyroid disorders
	Others: tumoural obstruction, fibrosing mediastinitis, chronic renal failure on dialysis

muscle cells into the small precapillary pulmonary arterioles, which normally lack a smooth muscle layer, and the abnormal presence of myofibroblasts is noted.[2] This proliferation of smooth muscle cells and myofibroblasts leads to luminal narrowing and a blunted ability to adequately dilate, with consequent increase in the upstream pressure. In later stage disease, areas of focal and disorganized neovascularization termed plexiform lesions develop.

In addition to medial smooth muscle proliferation, there are demonstrable perturbations in the extracellular matrix. Tenascin-C, a large matrix glycoprotein, promotes smooth muscle cell proliferation and is highly expressed within the medial layer of remodelled vessels in the animal and human studies.[3–5] Histologically therefore, in the lung there is a hyperproliferative narrowing of small vessels and focal areas of neoangiogenesis. Within these small vessels, there is an imbalance between vasodilatory and vasoconstrictive factors, either circulating or locally produced (Fig. 1). Vasoactive mediators converge on cytosolic calcium (Ca^{2+}), which is central to smooth muscle cell contraction. Smooth muscle Ca^{2+} can be influenced by a wide variety of mechanisms, including voltage-dependent Ca^{2+} channels, receptor-operated Ca^{2+} channels, store-operated Ca^{2+} channels, Ca^{2+} release from intracellular stores (primarily sarcoplasmic reticulum) and transport of Ca^{2+} through Ca^{2+} transporters. As the small vessels mostly contribute to resistance, there is an increase in the upstream pressure with pressure-induced changes in the larger vessels leading to reduced compliance in the proximal arteries.[6] The net effect of this is transmitted to

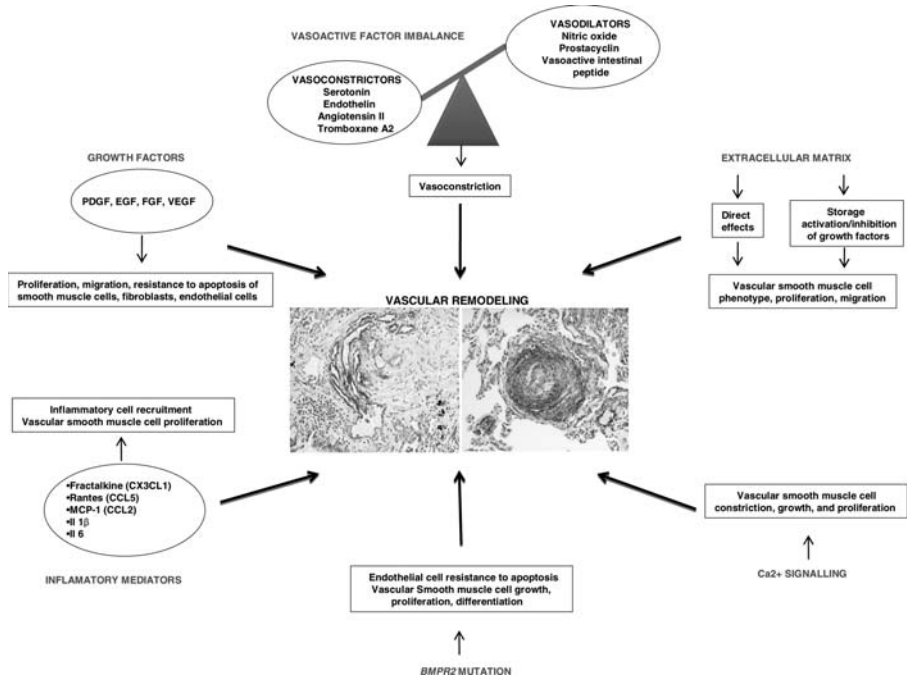

Fig. 1 Pathological mechanisms in PAH. PDGF, platelet-derived growth factor; EGF, epidermal-derived growth factor; FGF, fetal-derived growth factor; VEGF, vascular endothelial-derived growth factor; MCP-1, monocyte chemoattractant protein-1; IL, interleukin.

the right ventricle leading to right ventricular failure and eventually death. Despite the advancement of new classes of therapy targeting the pathologically dysregulated vasoreactivity, for most patients treatment is not curative and pulmonary hypertension still carries a poor prognosis, with, for example, a 1-year survival in a large French national registry of 88%.[7] In the modern treatment era, there is as yet no large long-term mortality figures published, but in the Papworth Hospital (one of the UK nationally designated specialist centres) our data suggests a 5-year survival of around 60%. This is comparable to the better known common cancers in the UK .[8]

The bone morphogenetic protein type-II receptor pathway

Arguably the most important advancement in pulmonary hypertension in recent years was the discovery of mutations in the transforming growth factor beta superfamily of receptors and downstream pathways, underlying a number of causes of pulmonary arterial hypertension (PAH).[9–11] These mutations all converge on the bone

morphogenetic protein type-II receptor (BMPR-II), which itself is mutated in around 70% of familial PAH and 20% in idiopathic disease.[12,13] Mutations in the endoglin receptor and activin-like receptor 1 (ALK-1), associated with hereditary haemorrhagic telangictasia,[10] have also been associated with a PAH phenotype in some families. Both of these receptors interact with BMPR-II at the cell surface to effect intracellular signalling, and are therefore working in part through the same pathway. In addition to a causative link to hereditary disease, it has also been demonstrated that BMPR-II is downregulated in the lungs and in cells from patients with idiopathic PAH without mutations in the BMPR-II gene.[14,15] This implies a broader relevance to dysfunction of BMPR-II to other forms of PAH. When trying to chase down the relevance of alterations in the BMPR-II function, there is a degree of complexity. BMPs are a diverse family of pleiotropic molecules and the ligand interactions differ for differing cell types. The response of vascular endothelial and smooth muscle cells to individual BMPs is dependent partly on the anatomical location of cells. For example while smooth muscle cells from large arteries are growth inhibited by BMPs 2 and 4, peripheral artery smooth muscle cells have enhanced proliferation.[15] That they have altered responses to ligands probably relates to their differing ultrastructure and contrasting function as conduit and resistance vessels. In other words, their function depends on where they are located and dictates why they might have different responses to the same stimulus. Endothelial cells meanwhile are in general protected from apoptosis by BMPs.[16] A potential paradigm for vascular remodelling can be derived from these observations, of reduced BMPR-II function in the endothelium promoting increased endothelial apoptosis, with subsequent compromise of the endothelial barrier allowing ingress of serum factors, which subsequently promote smooth muscle proliferation, myofibroblast proliferation, changes in the matrix and vascular remodelling. Therefore, it is clear that the BMPR-II pathway is central to the homeostasis of the pulmonary vasculature and that perturbations in its function can underlie the development of disease.

The emerging role of dysregulated immunity and inflammation

Pulmonary hypertension has long been associated with auto-antibody related diseases (Table 1), most notably scleroderma. In a recent French registry 15.3% of patients with PAH had a connective tissue

disease.[17] Human immunodeficiency virus (HIV) is also associated with pulmonary hypertension with a prevalence of around 0.5%.[18] The mechanism is not yet clear for these associations. In idiopathic PAH, there is a well-described association with auto-antibodies.[19] In addition, children with idiopathic PAH have increased frequencies of the MHC alleles DR3, DRw52, DQw2, which are classically associated with autoimmune disease.[20] In the PAH lung sections, an infiltration of leucocytes, macrophages and T cells has been described, particularly in plexiform lesions.[21] Although these associations are interesting evidence there remains a lack of clear mechanistic insight. Evidence is now however emerging from animal models pointing to possible mechanisms. In an athymic nude rat model, PAH developed under normoxic conditions with the addition of a vascular endothelial growth factor (VEGF) blocker, and could be ameliorated with immune reconstitution from spleen cells derived from euthymic rats.[22] Therefore, in this model an intact immune system is necessary for protection against PAH. In a mouse model of repeated antigen exposure, the mice developed muscularization of small arteries, and independently, depletion of CD4 cells and IL-13 both protected against this,[23] suggesting that although an intact pulmonary vasculature can be protective, hyperstimulation can also be deleterious and again this is seen in IL-6 overexpressing mice where there is the development of exaggerated right ventricular pressure and hypertrophy in response to hypoxia.[24]

In the traditional established animal models, including the monocrotaline-exposed rat model, disease progression is thought to rely centrally on inflammation, and schistosomiasis mouse model of vascular remodelling is also thought to generate vascular remodelling through inflammation.[25] It would therefore appear from animal models that the immune system is, perhaps unsurprisingly, implicated in repair and misrepair in vascular remodelling. In human studies inflammatory cytokines and chemokines such as IL-1β, IL-6, RANTES (regulated upon activation, normal T cell expressed and secreted, or CCL5), fractalkine and MIP-1α have all been demonstrated to be upregulated.[26–30] The interplay with lymphocyte recruitment and activation is again in evidence, for example with fractalkine (CX3CL1), which upregulates leucocyte recruitment, and RANTES, a chemoattractant for monocytes and T cells, both of which are upregulated in the vascular lesions of PAH.

Therefore in a broad variety of animal and human models of PAH there is compelling evidence that an intact immune system is necessary for the prevention of pulmonary hypertension. Dysregulated immunity is present in established diseases, and as a 'second hit' in conjunction with more traditional precipitants, results in worsened pathophysiology.

Advances in treatment

Current pharmacological treatment options

Until the 1990s, there was little in the way of good quality evidence-based therapy for PAH. Anti-coagulation was established on the basis of retrospective and uncontrolled studies.[31,32] Ca^{2+} channel blockers, potential vasodilators, are only convincingly effective in the 5% of patients who demonstrate a vasoactive response to acute vasoreactivity testing.[33,34] The introduction of epoprostenol heralded a new era in randomized controlled trials of therapy in PAH.[35,36] All of the current nine therapies that are fully licensed or under regulatory review were introduced to target mechanisms related to vasoactivity, via the nitric oxide, the prostacyclin and the endothelin pathways (Fig. 2). This approach has had undoubted success in reducing morbidity and improving quality of life, but although there has been an impact in improving mortality, this remains modest with a recent meta-analysis

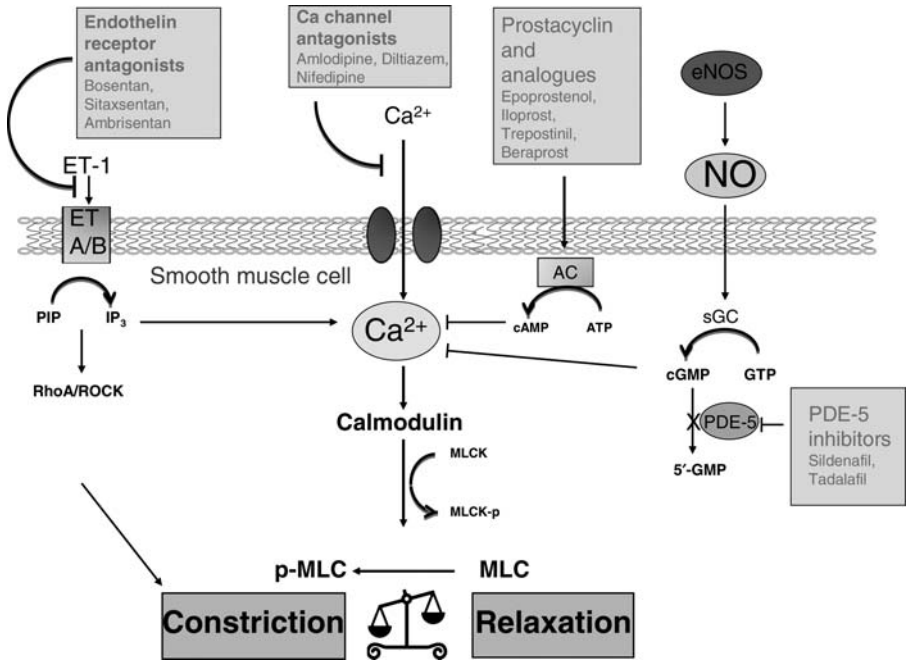

Fig. 2 Mechanisms current therapies utilize to effect vasodilatation in PAH. AC, adenylate cyclase; ATP, adenosine triphosphate; cAMP, cyclic adenosine monophosphate; c-GMP, cyclic guanosine monophosphate; eNOS, endothelial nitric oxide synthase; ET-1, endothelin-1; ET A/B, endothelin receptors A/B; GTP, guanosine triphosphate; IP_3, inositol 1,4,5-trisphosphate; MLC, myosin light chain; MLCK, MLC kinase; MLC-p, MLC phosphatase; PDE-5, phosphodiesterase 5; p-MLC, phospho-MLC; PIP, phosphatidylinositol; NO, nitric oxide; RhoA, Ras homolog gene family, member A; ROCK, RhoA kinase; sGC, soluble guanylate cyclase.

suggesting a 43% improvement in short-term mortality[37] but little in the way of long-term prospective data. Of note this meta-analysis grouped all of the different vasoactive therapeutic classes together as the numbers are small for each individual therapy. We therefore have no clear data of superiority of any particular drug, and good head-to-head randomized and blinded trials have not been performed. In addition the role of combination therapy is still to be clarified. Although there is on-going work in improving the pulmonary selectivity and strength in novel vasoactive therapies, attention is now shifting to developing therapies, which act via alternative mechanisms in attempts to broaden the therapeutic options.

Anti-proliferative agents

As one of the characteristic pathological features of PAH is the heightened proliferation of smooth muscle in the small resistance arteries, a great deal of interest is currently focused on the concept of reversing this process. In some ways this is essentially best understood by comparing the process to a cancer paradigm. PAH has parallels with malignant disease, in particular the abnormal proliferation of vascular cells and in plexiform lesions, monoclonal endothelial cell expansion.[38] In particular plexiform lesions have been suggested to share features with malignant cells.[39] In animal models of PAH blocking growth factors such as platelet-derived growth factor (PDGF) and VEGF can reverse PAH.[40,41] The first growth factor inhibitor to be tested, the tyrosine kinase inhibitor imatinib, was initially designed to block breakpoint cluster region Abelson murine leukemia viral oncogene homolog in the treatment of chronic myeloid leukaemia.[42] The subsequent demonstration that it had off-target effects on other tyrosine kinase receptors such as the PDGF and cKit receptors, made it an attractive anti-proliferative agent in a number of settings.[42] A 6-month phase II safety study in PAH has demonstrated some improvement in pulmonary haemodynamics but is as yet unpublished. Phase III trials are currently about to enroll. No other therapies have yet reported phase II trials but there is growing interest in this approach.

The next generation of vasodilators

Work continues on augmenting vasodilatation, to date the only convincing therapeutic strategy to yield results. The most attractive novel candidate pathway centres around soluble guanylate cyclase (sGC), which

mediates vasoactivity through cyclic guanosine monophosphate and control of intracellular Ca^{2+} stores (Fig. 2). Soluble GC stimulators and activators partially inhibit vascular remodelling in animal models of PAH.[43–45] The stimulators augment the nitric oxide (NO) effects on the enzyme, while the activators act via NO independent mechanisms and are therefore particularly of interest. The first human trial of an sGC stimulator, Riociguat (compound BAY63–2521), in 19 subjects demonstrated a significant improvement acutely in pulmonary haemodynamic parameters and cardiac index in patients with PAH in a dose-dependent manner. Of interest the effects were greater than those observed with inhaled NO.[46] Other vasodilators such as vasoactive intestinal peptide (VIP), are also potential targets. Again clinical trial data on VIP in humans are currently unpublished.

Stem/progenitor cells in PAH

There is controversy over the role of endothelial progenitor cells (EPCs) in vascular injury and repair. In pulmonary hypertension the initial studies involving putative EPCs conducted were predominantly animal model-based therapeutic studies. These demonstrated efficacy of what were initially thought to represent EPCs, infused into animal models of disease.[47–50] Zhao *et al.* also additionally transfected EPCs with eNOS, suggesting that regardless of the role EPCs were playing they could potentially be used for gene transfer. There have been two small non-randomized trials in humans also demonstrating a short-term improvement in symptoms and haemodynamics,.[51,52] In the majority of these trials, what were termed EPCs have subsequently been demonstrated to be circulating monocytes without proliferative potential and therefore not technically progenitor cells.[53] Further confusing the issue, numerous groups have demonstrated putative progenitor cells, which appear to be functionally defective in patients with PAH,[54–56] and potentially even contribute to the formation of plexiform lesions.[54,57] There is additionally good evidence that mesenchymal progenitor cells are also upregulated in tissue from animal models and human disease with a potential causal link to matrix deposition.[58–61] Stem cells remain an interesting potential avenue for novel treatment but more work needs to be done to clarify what exactly their role is in pathology before the next generation of trials is planned. Any consideration of cell-based therapeutic strategies will require intensive investment in optimized infrastructure for stem-cell culturing conditions, to mitigate the technological difficulties in complying with good manufacturing practice.

The future of treatment

The last 10 years has seen a change in focus to the pathological site of disease, the small resistance vessels. This has undoubtedly been a success, if not as dramatic as hoped. Within these vessels the role dysregulated angiogenesis plays is still hotly debated.[62] Whether manipulation of angiogenesis will yield therapeutic results is unclear, although the cancer and cardiovascular fields are well developed in anti- and pro-angiogenic drug development. It is therefore entirely possible that the process of translating on-going research into tangible treatments could be much faster that expected. Ultimately the focus on the small vessels has ignored the fact that patients die of 'end-organ damage', in other words failure of the right ventricle. It is likely that at some point the issue of supporting and augmenting the function of the right ventricle will need to be re-addressed. Also being widely discussed is the role for pharmacogenomics, which may lead to better tailored therapy, although this is likely some distance off. It is clear, however that we are still in an exciting growth phase of research and treatment development, and the prospects for patients in the future continue to improve. It is entirely plausible that in the next two decades we will see that PAH being turned into a chronic and manageable disease.

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