Heart, Lung and Circulation (2014) **23,** 595–601
1443-9506/04/$36.00
http://dx.doi.org/10.1016/j.hlc.2014.02.007

REVIEW

Endothelial Progenitor Cells and Pulmonary Arterial Hypertension

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Received 1 February 2014; accepted 17 February 2014; online published-ahead-of-print 6 March 2014

Pulmonary arterial hypertension (PAH) is a progressive disease characterised by lung endothelial cell dysfunction and vascular remodelling. A number of studies now suggest that endothelial progenitor cells (EPCs) may induce neovascularisation and could be a promising approach for cell based therapy for PAH. On the contrary EPCs may contribute to pulmonary vascular remodelling, particularly in end-stage pulmonary disease. This review article will provide a brief summary of the relationship between PAH and EPCs, the application of the EPCs to PAH and highlight the potential clinical application of the EPCs cell therapy to PAH.

Keywords Pulmonary arterial hypertension (PAH) • Endothelial progenitor cells (EPCs) • Cell therapy • Vascular remodelling • Pulmonary disease

Introduction

PAH represents a variety of cardiovascular and systemic diseases and is defined as an increase in arterial pressure above 25 mm Hg [1]. It is characterised by cellular and structural changes in the walls of pulmonary arteries, resulting in endothelial dysfunction and vascular remodelling [2]. Endothelial dysfunction affects levels of vasoactive mediator balance resulting in vasoconstriction. Excessive pulmonary arterial smooth muscle cell proliferation and impaired apoptosis in pulmonary vessels can lead to structural remodelling. Ultimately injury to the endothelial cells of the pulmonary arteries is central to the subsequent development of lumen-obliterative lung vascular lesions [3].

Current pharmacological treatment of PAH includes prostacyclin based therapy to improve vasodilation and the use of endothelin-1 receptor antagonists to reduce vasoconstriction, also inhibitors of phosphodiesterase type 5, such as sildenafil which can enhance the activity of nitric oxide (NO) through the breakdown of cyclic guanosine monphosphate [4]. Other emerging pharmacological candidates include soluble guanylyl cyclase, serotonin receptor 2B and Rho kinase inhibitors. However a truly restorative therapy has not been achieved. Recently increased attention has been given to endothelial progenitor cells (EPCs). EPCs are thought to be important in maintaining vascular homeostasis by homing to sites of vascular injury and regenerating blood vessels [5]. They can be mobilised from the bone marrow, traffic to a site of injury and reside locally in the lung [6,7]. There is growing interest in the concept of EPCs therapy for the treatment of PAH.

Influencing factors and the pathology of PAH

PAH is a progressive, debilitating lung disorder, and ultimately lethal disease with a mortality rate of 50% in five years [8]. Several contributing factors include mitochondrial

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dysfunction [9,10], the enhanced presence of alpha-SM-actin + cells, inflammation [11], changes in the right ventricle, metabolic dysfunction [12–14], expression of endothelial GATA-6 [15], MicroRNAs [16,17], oxidative stresses [18–20], antioxidant enzyme deficiencies [21], angiogenesis, and EPCs play an important role in the development of PAH [22]. Pathways involved in pulmonary vascular remodelling include notch signalling pathways [23]. Serotonin (5-HT) and its receptors 5-HT (2B) have been identified as contributors to the PAH pathogenesis [2,24]. Mast cells may also contribute to pulmonary vascular pathologic processes [25]. A significant finding was the discovery that familial PAH based disease is associated with mutations in the bone morphogenic protein receptor type 2(BMPR2) [26–29]; up to 80% of PAH and 20% of spontaneous PAH is associated with a BMP receptor mutation. Other mutated genes linked to PAH include activin-like kinase-type 1(ALK1) [30] and endoglin (ENG) [31]. As disease progresses bone marrow-derived or circulating progenitor cells have also been shown to be recruited to sites of vascular injury and to assume both endothelial and SM-like phenotypes [32].

PAH is characterised by pathological features in the small peripheral pulmonary arteries which include intimal thickening and fibrosis, medial hypertrophy and changes in the adventitia [33], excessive vascular cell growth, infiltration of inflammatory mediators. Hallmark plexiform lesions are commonly seen in late disease and are observed in the walls of pulmonary arteries associated with proliferating endothelial cells [34]. The evident endothelial dysfunction leads to enhanced proliferation of pulmonary arterial SMCs (PASMCs) and ultimately vessel occlusion accompanied by ECM deposition in the external elastic lamina. Interestingly these proliferating PASMCs appear to show increased glycolysis which may contribute to their resistance to apoptosis [35].

These combined cellular effects lead to a widespread narrowing or obliteration of the pulmonary artery resulting in increased pulmonary vascular resistance [36]. These effects are likely to be mediated by a combination of endothelial dysfunction, pulmonary endothelial barrier disruption, fibroblast and smooth muscle cell activation, and circulating progenitor cells recruitment [37,38].

PAH has been linked to myeloid abnormalities, characterised by high circulating CD34 (+) and CD133 (+) proangiogenic progenitor cells, and endothelial cells have shown a pathologic expression of hypoxia-inducible factor 1 alpha (HIF-1alpha). Upregulation of HIF-1 alpha has been shown to contribute to endothelial cell dysfunction in PAH, and can be related to disease severity [39–41], this leads to a high production of myeloid-activating factors such as erythropoietin, stem cell factor (SCF), and hepatocyte growth factor (HGF) in PAH blood [42]. These factors may recruit proangiogenic progenitor cells to the pulmonary circulation where they could contribute to angiogenic remodelling of the vessel wall [43,44]. It has been reported that PAH, in contrast to chronic thromboembolic pulmonary hypertension (CTEPH), is associated with markers of vascular injury (circulating endothelial cells, soluble E-selectin and soluble vascular cell adhesion molecule (sVCAM)) but not with markers of remodelling (EPCs, CD34 (+) CD133 (+)) cells and VEGF [45]. The role of EPCs in PAH pathology has been demonstrated where cell numbers and functional capacity were impaired in patients with IPAH [46]. Furthermore Anjum et al. found that fewer EPCs in PAH patients may contribute to the pulmonary vascular pathology [47].

Pharmacological treatment of PAH

With vasoconstriction largely contributing to the pathophysiology of PAH, the use of vasodilators is a common treatment approach. There are at least three classes of drug therapy for PAH: *prostaglandins* (iloprost, treprostinil, epoprostenol, prostacyclins, selexipag and APD-811), *endothelin receptor antagonists* (bosentan, ambrisentan, sitaxsentan, macitentan and tezosentan), and *phosphodiesterase-5 inhibitors* (sildenafil, tadalafil, vardenafil and udenafil) [4]. Prostacyclin receptor agonists such as the prodrug selexipag, activate the IP receptor leading to increased vasodilation. Soluble guanylate cyclase stimulators (such as Riociguat) enhance GMP synthesis leading to vasodilation. In an animal model of PAH, Riociguat treatment reversed muscularisation of pulmonary arterioles and right ventricular hypertrophy [48]. Inhibition of the Rho-Kinase pathway is also an attractive target in that increased cytosolic calcium promotes proliferation of PASMCs through indirect inhibition of the myosin light chain phosphatase [49]. Fasudil, an example of a Rho-kinase inhibitor, has shown a reduction in PA pressure in patients with PAH [50].

The role of EPCs in vasculogenesis, vascular repair and regeneration

The clinical experience to date suggests that EPCs may contribute to vascular repair in PAH. The seminal discovery by Asahara demonstrated a population of CD34+ve or KDR positive cells which were shown to be involved in adult neovascularisation. These cells, termed endothelial progenitor cells, are mobilised from the bone marrow in response to stimuli. Their ability to home to an injured region via the SDF1alpha/CXCR4 system has shown them to play a role in post ischaemic angiogenesis [51]. Studies have also found that levels of circulating EPCs are directly related to peripheral and coronary endothelial function [52]. Similarly a reduction in circulating EPCs numbers has been associated with pneumonia and chronic lung disease. Endothelium maintenance and restoration of normal endothelial cell function are governed by a complex physiological procedure in which EPCs play a significant role. EPCs are capable of maintaining, generating, and replacing terminally differentiated cells within their own specific tissue as a consequence of physiological cell turnover or tissue damage due to injury [53]. Circulating EPCs may represent an endogenous repair mechanism for endothelial dysfunction. Or, as some studies