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Abstract: Primary pulmonary hypertension (PPH) is a fatal cardiovascular disease characterized by increased pulmonary vascular resistance and progressive right heart failure. It is associated with a very high morbidity and mortality rate. The therapeutic effects of current pharmacological management of PPH are limited. Recent studies have demonstrated that endothelial dysfunction and cell loss play a critical role in the pathogenesis of PPH. Emerging evidence also shows that circulating endothelial progenitor cells instigate new vessel formation via vasculogenesis and revascularization, and provide ongoing endothelial repair by homing to site of endothelial damage. We hypothesized that autologous endothelial progenitor cells transplantation may be a feasible adjunctive therapeutic option for PPH.

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Autologous Endothelial Progenitor Cells Transplantation for the Therapy of Primary Pulmonary Hypertension

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Abstract

Primary pulmonary hypertension (PPH) is a fatal cardiovascular disease characterized by increased pulmonary vascular resistance and progressive right heart failure. It is associated with a very high morbidity and mortality rate. The therapeutic effects of current pharmacological management of PPH are limited. Recent studies have demonstrated that endothelial dysfunction and cell loss play a critical role in the pathogenesis of PPH. Emerging evidence also shows that circulating endothelial progenitor cells instigate new vessel formation *via* vasculogenesis and revascularization, and provide ongoing endothelial repair by homing to site of endothelial damage. We hypothesized that autologous endothelial progenitor cells transplantation may be a feasible adjunctive therapeutic option for PPH.

Key words: Pulmonary hypertension, endothelial progenitor cells, transplantation.

Introduction

Primary pulmonary hypertension (PPH) is a progressive disease of unknown aetiology manifested by increased pulmonary vascular resistance and progressive right heart failure ^[1]. The pulmonary arteries in PPH are characterized by intimal fibrosis, medial hypertrophy, adventitial proliferation, and obliteration of small arteries ^[2]. PPH responds poorly to conventional therapeutic agents such as anticoagulants, diuretics, and calcium-channel blockers. Data from the US National Institutes of Health (NIH) registry on PPH show that PPH is a fatal disease in which most of the patients die within 2 to 3 years from the diagnosis ^[3,4]. The 1-, 2- and 5-year survival rate is 68%, 48% and 34%, respectively ^[3,4]. After lung and heart-lung transplantation, the 3- and 5-year survival is approximately 55% and 45%, respectively ^[5]. Although new classes of drugs such as prostanoids ^[6], endothelin receptor antagonists ^[7] and type 5 phosphodiesterase inhibitors ^[8] has been shown to exert favourable effects on PPH patient's haemodynamics, exercise capacity and survival in patients with severe PPH, and the long-term prognosis of the disease is still poor. Therefore, it is of paramount importance to develop new therapeutic strategies for this devastating disease.

Our understanding of the pathogenetical and pathobiologic mechanisms underlying PPH has progressed rapidly over the past few years. Current evidence strongly suggests a central role for endothelial dysfunction and cell loss in the initiation and progression of PPH. Endothelial progenitor cells are a subset of pluripotent "stem cells" derived from the bone marrow that can circulate in the blood.

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Release of endothelial progenitor cells from the marrow occurs in response to chemokines produced as a result of remote injury. Recent studies have demonstrated that circulating endothelial progenitor cells not only have the potential to instigate new vessel formation *via* angiogenesis and neovascularisation, but also have the potential to provide ongoing endothelial repair by homing to site of endothelial damage^[9,10]. Autologous endothelial progenitor cells transplantation may be a feasible adjunctive therapeutic option for PPH.

Endothelial dysfunction in pulmonary hypertension

Endothelial integrity and normal function are indispensable for the preservation of health. The estimated 1 to 6×10^{13} cells of the endothelial monolayer form an approximately 1-kg heavy “organ” in an adult human, and have a central role in the control of vascular tone, permeability, blood flow, coagulation, thrombolysis, inflammation, tissue repair, and growth^[11,12].

It is recognised that a number of stimuli, including shear stress from increased pulmonary blood flow, viral infection (HIV), and alveolar hypoxia, may potentially injure endothelium in genetically predisposed individuals. Injury to the endothelium leads to apoptosis of the usually quiescent cells, destabilisation of the pulmonary vascular intima, and disordered endothelial cell proliferation. Pulmonary endothelial dysfunction results in an altered production of vasodilators, such as nitric oxide and prostacyclin^[13,14], and overexpression of vasoconstrictors, such as thromboxane A₂ and endothelin-1^[15,16], which lead to pulmonary vasoconstriction.

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The loss of endothelial barrier integrity permit the extravasation of factors that stimulate smooth muscle cell (SMC) production of a vascular serine elastase ^[17], resulting in the liberation of matrix-bound SMC mitogens, such as fibroblast growth factor, and initiation of growth signals to medial smooth muscle cells via degradation of matrix elements ^[18]. In addition, vascular smooth muscle and adventitia may appear adaptive hypertrophy in response to the increase of luminal pressure. These phenomena constitute the pulmonary vascular remodeling.

Endothelial dysfunction increases the production of various pro-thrombotic and decreases the production of anti-thrombotic substances, resulting in a hypercoagulable state ^[19]. Platelet activation may also take place when platelets come in contact with the subendothelial structures ^[20]. Levels of serotonin, plasminogen activator inhibitor, and fibrinopeptide A are elevated and thrombomodulin levels are decreased ^[21,22]. Thrombosis narrows the pulmonary vessel lumen, which may help propagate the changes of PPH.

Wright et al. ^[23] recently demonstrated an increased expression of 5-lipoxygenase (5-LO) and 5-LO activating protein (FLAP); both are mediators of leukotriene synthesis in the pulmonary endothelium of patients with PPH. Elevated levels of macrophage inflammatory protein-1 α , interleukin-1 β , interleukin-6 and P-selectin are also found in patients with severe PPH ^[24,25].

These evidences suggest endothelial dysfunction play an integral role in mediating the structural changes in the pulmonary vasculature.

Role of endothelial progenitor cells in vasculogenesis

There are two different processes in the formation of new blood vessels. The first process, vasculogenesis, implies the de novo organization of endothelial cells into vessels in the absence of any pre-existing vascular system ^[26], and is believed until recently only occurs in the early embryo. The second process, termed angiogenesis, is defined as the formation of new vessels by sprouting from preexisting blood vessels ^[27]. The discovery of endothelial progenitor cells in adults ^[28] has led to the new concept that vasculogenesis and angiogenesis may occur simultaneously. Accumulating evidence suggests that vasculogenesis plays an important role in postnatal neovascularization of adult ischaemic tissues ^[29]. Human endothelial progenitor cells were initially characterized by the expression of the vascular endothelial growth factor (VEGF) receptor 2 (VEGF R2; Flk-1) and a haematopoietic marker such as CD133 ^[29]. Endothelial progenitor cells provide a circulating pool of cells that could form a cellular patch at the site of denuding injury or serve as a cellular reservoir to replace dysfunctional endothelium ^[30]. Although the mechanism is poorly understood, injected endothelial progenitor cells have the capacity to reach a neoangiogenic site there they differentiate into endothelial cells. Infusion of *ex vivo* expanded endothelial progenitor cells was shown to augment capillary density and neovascularization of ischaemic tissue ^[31, 32]. Furthermore, endothelial progenitor cells have a favorable survival and a better response toward angiogenic growth factors compared with mature endothelial cells ^[33].

Endothelial progenitor cell transplantation in PPH

Recently, Noritoshi Nagaya^[34] described the effect of endothelial progenitor cell transplantation on PPH. They obtained endothelial progenitor cells from cultured human umbilical cord blood mononuclear cells, then injected intravenously into immunodeficient nude PPH rats. Two weeks later, pulmonary vascular resistance was significantly lower in the endothelial progenitor cell group (-16%) than in the control group^[34]. Another animal study indicated transplantation of endothelial progenitor cells prevented further progression of PPH^[35]. These results suggest endothelial progenitor cell transplantation may be a feasible adjunctive therapeutic option for PPH. We hypothesize that the mechanism of attenuation of pulmonary pressure is that injected endothelial progenitor cells contribute to the formation of new blood vessels and thus increase the area of pulmonary vessel bed, replacing dysfunctional endothelium and maintaining the functional integration of vessel endothelium, which may restore the balance of vasoactive substance, ameliorate vessel remodeling and prevent the formation of thrombus in site.

Our hypothesis has been partly tested in our recent pilot studies. After receiving approval from the Institutional Review Board of the First Hospital of Zhejiang University, we divided four beagle dogs into three groups: sham, control, and transplantation. Pulmonary artery pressure was obtained by a Swan-Ganz float catheter inserted to the pulmonary artery through the right jugular vein. Dehydromonocrotaline (DHMC, 3mg/kg) was administered via the right atrium to establish pulmonary hypertension model. Six weeks later, pulmonary artery pressure

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was re-evaluated. Ex vivo-expanded, autologous endothelial progenitor cells originated from peripheral blood were then injected into pulmonary artery. DHMC was prepared from Monocrotaline according to Mattocks et al ^[36] and endothelial progenitor cells were expanded according to the methods reported by Asahara et al ^[37]. Haemodynamic changes were reevaluated four weeks after the injection of endothelial progenitor cells. In the sham animal, there was no change in the mean pulmonary artery pressure (Table 1). In the control group, the pulmonary artery pressure was increased from 21 to 32 mm Hg (Table 1). In the transplantation group, however, the mean pulmonary artery pressure remained largely unchanged (Table 1).

Although this pilot study did not have sufficient statistical power to prove or disapprove the therapeutic effect of endothelial progenitor cell transplantation, a larger study may well be able to demonstrate the effect of the transplantation.

Table 1. Mean pulmonary artery pressure (mm Hg) before and after endothelial progenitor cell transplantation.

Group	Baseline	After DHMC injection	After transplantation
Sham (n=1)	11	12	12
Control (n=1)	13	21	32
Transplantation (n=2)	11,13	20, 22	19, 27

Reference

1. Rubin LJ. Current concepts: primary pulmonary hypertension. *N Engl J Med* 1997; 336:111–117.
2. Chazova I, Loyd JE, Zhdanov VS, et al. Pulmonary artery adventitial changes and venous involvement in primary pulmonary hypertension. *Am J Pathol* 1995; 146:389–397.
3. Rich S, Dantzker DR, Ayres SM, et al. Primary pulmonary hypertension: a national prospective study. *Ann Intern Med* 1987; 107:216–230.
4. D'alonzo GE, Barst RJ, Ayres SM, et al. Survival in patients with primary pulmonary hypertension: results of a national prospective registry. *Ann Intern Med* 1991; 11:343–349.
5. Hertz MI, Taylor DO, Trulock EP et al. The registry of the International Society for Heart and Lung Transplantation: Nineteenth Official Report 2002. *J Heart Lung Transplant* 2002; 21:950–970.
6. Galie N, Manes A, Branzi A. Prostanoids for pulmonary arterial hypertension. *Am J Respir Med* 2003; 2:123–137.
7. Galie N, Manes A, Branzi A. The endothelin system in pulmonary arterial hypertension. *Cardiovasc Res* 2004; 61:227–237.
8. Sastry BKS, Narasimhan C, Reddy NK et al. Clinical efficacy of sildenafil in primary pulmonary hypertension: A randomized, placebo-controlled, double-blind, crossover study. *J Am Coll Cardiol* 2004; 43:1149–1153.

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9. Shi Q, Rafii S, Wu MH, et al. Evidence for circulating bone marrow-derived endothelial cells. *Blood* 1998; 92:362-367.
10. Takahashi T, Kalka C, Masuda H, et al. Ischemia and cytokine-induced mobilization of bone marrow-derived endothelial progenitor cells for neovascularization. *Nat Med* 1999; 5:434-438.
11. Cines DB, Pollak ES, Buck CA, et al. Endothelial cells in physiology and in the pathophysiology of vascular disorders. *Blood* 1998; 91:3527–3561.
12. Rubanyi GM. The role of endothelium in cardiovascular homeostasis and diseases. *J Cardiovasc Pharmacol* 1993; 22(Suppl):S1–S4.
13. Christman BW, McPherson CD, Newman JH, King GA, Bernard GR. An imbalance between the excretion of thromboxane and prostacyclin metabolites in pulmonary hypertension. *N Engl J Med* 1992; 327:70–75.
14. Giaid A, Saleh D. Reduced expression of endothelial nitric oxide synthase in the lungs of patients with pulmonary hypertension. *N Engl J Med* 1995; 333:214–221.
15. Humbert M, Morrell NW, Archer SL, et al. Cellular and molecular pathobiology of pulmonary arterial hypertension. *J Am Coll Cardiol* 2004; 43:Suppl 12:13S-24S.
16. Giaid A, Yanagisawa M, Langleben D, et al. Expression of endothelin-1 in the lungs of patients with pulmonary hypertension. *N Engl J Med* 1993; 328:1732–1739.
17. White CR, Darley-Usmar V, Berrington WR, et al. Circulating plasma xanthine oxidase contributes to vascular dysfunction in hypercholesterolemic rabbits. *Proc Natl Acad Sci* 1996; 93:8745–8749.

18. Cowan KN, Jones PL, Rabinovitch M. Elastase and matrix metalloproteinase inhibitors induce regression, and tenascin-C antisense prevents progression, of vascular disease. *J Clin Invest* 2000; 105:21–34.
19. Bombeli T, Mueller M, Haerberli A. Anticoagulant properties of the vascular endothelium. *Thromb Haemost* 1997; 77:408–423.
20. Ruf A, Morgenstern E. Ultrastructural aspects of platelet adhesion on subendothelial structures. *Semin Thromb Hemost* 1995; 21:119–122.
21. Frank H, Mlczoch J, Huber K, et al. The effect of anticoagulant therapy in primary and anorectic drug-induced pulmonary hypertension. *Chest* 1997; 112:714–721.
22. Welsh CH, Hassell KL, Badesch DB, et al. Coagulation and fibrinolytic profiles in patients with severe pulmonary hypertension. *Chest* 1996; 110:710–717.
23. Wright L, Tuder RM, Wang J, et al. 5-Lipoxygenase and 5-lipoxygenase activating protein (FLAP) immunoreactivity in lungs from patients with primary pulmonary hypertension. *Am J Respir Crit Care Med* 1998; 157:219–229.
24. Fartoukh M, Emilie D, Le Gall C, et al. Chemokine macrophage inflammatory protein-1 mRNA expression in lung biopsy specimens of primary pulmonary hypertension. *Chest* 1998; 114:50S–51S.
25. Humbert M, Monti G, Brenot F, et al. Increased interleukin-1 and interleukin-6 serum concentrations in severe primary pulmonary hypertension. *Am J Respir Crit Care Med* 1995; 151:1628–1631.
26. Risau W, Flamme I. Vasculogenesis. *Annu Rev Cell Dev Biol* 1995; 11:73.

27. Folkman J. Clinical applications of research in angiogenesis. *N Engl J Med* 1995; 333:1757.
28. Asahara T, Murohara T, Sullivan A, et al. Isolation of putative progenitor endothelial cells for angiogenesis. *Science* 1997; 275:964-967.
29. Rafii S, Lyden D. Therapeutic stem and progenitor cell transplantation for organ vascularization and regeneration. *Nat Med* 2003; 9:702–712.
30. Walter DH, Rittig K, Bahlmann FH, et al. Statin therapy accelerates reendothelialization: a novel effect involving mobilization and incorporation of bone marrow-derived endothelial progenitor cells. *Circulation* 2002; 105:3017-3024.
31. Kawamoto A, Gwon HC, Iwaguro H, et al. Therapeutic potential of ex vivo expanded endothelial progenitor cells for myocardial ischemia. *Circulation* 2001; 103:634–637.
32. Tateishi-Yuyama E, Matsubara H, Murohara T, et al. Therapeutic angiogenesis for patients with limb ischaemia by autologous transplantation of bone-marrow cells: a pilot study and a randomised controlled trial. *Lancet* 2002; 360:427–435.
33. Bompais H, Chagraoui J, Canron X, et al. Human endothelial cells derived from circulating progenitors display specific functional properties as compared to mature vessel wall endothelial cells. *Blood* 2004; 103:2577-2584.
34. Nagaya N, Kangawa K, Kanda M, Uematsu M. Hybrid cell–gene therapy for pulmonary hypertension based on phagocytosing action of endothelial progenitor cells. *Circulation* 2003; 108; 889-895.

35. Yidan D, David W, Yupu D, et al. Rescue of monocrotaline-induced pulmonary arterial hypertension using bone marrow-derived endothelial-like progenitor cells efficacy of combined cell and eNOS gene therapy in established disease. *Circ Res* 2005; 96; 442-450.
36. Mattocks AR, Jukes R, Brown J. Simple procedures for preparing putative metabolites of pyrrolizidine alkaloids. *Toxicon* 1989; 27:561-567.
37. Asahara TH, Masuda T, Takahashi C, et al. Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physiological and pathological neovascularization. *Circ Res* 1999; 85:221–228.