Stem and progenitor cell therapy for pulmonary arterial hypertension: effects on the right ventricle (2013 Grover Conference Series)

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Abstract: In experimental animals and in patients with pulmonary arterial hypertension (PAH), a wide spectrum of structural and functional conditions is known that may be responsible for the switch of a state of "compensated" right ventricular (RV) hypertrophy to a state of RV failure. In recent years, therapy with differentiated cells, endothelial progenitor cells, and mesenchymal stem cells has been shown to cause partial or complete reversal of pathological characteristics of PAH. The therapeutic effects of stem or progenitor cell therapy are considered to be (1) paracrine effects from stem or progenitor cells that had engrafted in the myocardium (or elsewhere), by compounds that have anti-inflammatory, antiapoptotic, and proangiogenic actions and (2) unloading effects on the right ventricle due to stem or progenitor cell–induced decrease in pulmonary vascular resistance and decrease in pulmonary artery pressure.

Keywords: pulmonary arterial hypertension, right ventricular hypertrophy, right ventricular failure, cell therapy, stem cells, mesenchymal stem cells, progenitor cells, endothelial progenitor cells, homing, engraftment, differentiation, paracrine factors.

Pulm Circ 2015;5(1):73-80. DOI: 10.1086/679701.

Pulmonary arterial hypertension (PAH) is associated with right ventricular (RV) hypertrophy (RVH) or RV failure (RVF), considered to be the result of RV pressure overload. Pharmacotherapy, gene therapy, stem and progenitor cell therapy, and combinations of these treatment modalities have been shown to treat PAH, by lowering pulmonary artery pressure, lowering RV weight, and relieving RVF, thereby improving exercise capacity and life span.[1] Here we review the evidence that, in animals with PAH, stem and progenitor cell therapy leads to lower RV weight and relief of RVF by mechanisms that can be ascribed to (1) lowering of RV afterload and (2) direct effects of the stem and progenitor cells on RV myocardial structure and function.

RVH DUE TO PAH-INDUCED RV OVERLOAD: PATHOLOGICAL OR PHYSIOLOGICAL HYPERTROPHY?

Originally, left ventricular (LV) hypertrophy (LVH) secondary to pressure overload was considered to be "compensatory,"[2] but several studies have clearly demonstrated that LVH is a risk factor and has unfavorable prognostic implications.[3-5] On the other hand, the athlete's heart often implies the presence of LVH, but this LVH is considered an adaptation to volume overload that occurs during long-lasting rowing, running, and cycling.[6] To date, the healthy aspects of exercise training have been documented firmly and are not restricted to healthy individuals, such as athletes, but even to patients with heart failure.[7,8]

The two main forms of myocardial hypertrophy are often termed "pathological" hypertrophy, as occurs secondary to pressure overload, and "physiological" hypertrophy, as occurs in the athlete's heart. Pathological and physiological hypertrophy have been characterized in detail,[9-13] and the most significant differences are found in the membrane receptor and signaling pathways that convey the message to the nucleus, where hypertrophy is effectuated with or without changes in gene expression. In pathological hypertrophy, Ca^{2+}-dependent calcineurin activation and calcineurin-induced dephosphorylation of

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Submitted January 29, 2014; Accepted August 25, 2014; Electronically published January 21, 2015.

nuclear factor of activated T cells (NFAT) trigger gene transcription in the nucleus, leading to multiple changes in gene expression, including expression of "fetal" genes such as atrial natriuretic peptide (ANP), β-myosin heavy chain (β-MHC), and α-skeletal-actin. The β-adrenergic receptor density is decreased, and this receptor is desensitized.[14] In addition, during the development of cardiac hypertrophy, a mismatch between the number of capillaries and the size of cardiomyocytes can lead to ischemia and contractile dysfunction.[15] In physiological hypertrophy, signalling through Akt, mTOR, p70S6 kinase, and S6 activates gene transcription in the nucleus, which is hardly associated with changes in gene expression. The β-adrenergic receptor density and capillary density in the myocardium remain unchanged, compared to those in controls.[16]

The transition from RVH to RVF is dependent on a large number of known and unknown conditions and factors. A new player in this field is the family of microRNAs of which the expressions are changing during the process of RV remodeling.[17] MicroRNAs are small, noncoding RNAs that are emerging as crucial regulators of cardiac remodeling in LVH and LV failure.[18,19]

ADMINISTRATION OF EXOGENOUS STEM OR PROGENITOR CELLS FOR LUNG REPAIR

Cytotherapy with stem and progenitor cell types is a novel approach to treat several lung diseases. This approach is based on the concept that stem and progenitor cells may help to regenerate and repair pulmonary vasculature.[20] Stem cells are undifferentiated cells that display self-renewal, clonogenicity, and multipotency. Mesenchymal stem cells (MSCs) are cells that adhere to plastic, express surface cell markers such as CD73, CD90, and CD105; lack surface expression of CD11b, CD14, CD19, CD34, CD45, CD79α, and HLA-DR; and are able to differentiate into multiple cell types, such as adipocytes, osteocytes, and chondrocytes, in vitro.[21] MSCs have the ability and tendency to preferentially migrate to injured lung tissue, where they secrete angiogenic (e.g., vascular endothelial growth factor [VEGF], Ang-1), antiapoptotic (Bcl-2), and anti-inflammatory factors (e.g., interferon-γ, interleukin-10, VEGF, hepatocyte growth factor [HGF]).[22] They also exert immunomodulatory effects by direct cell-to-cell contact.[23,24] These properties, in combination with their low immunogenicity and their ability to evade clearance by the immune system of the host,[25] make MSCs an ideal candidate in therapies for a variety of lung diseases.

Progenitor cells are committed to a lineage and differentiate into a specific cell or cell types. Compared to stem cells, progenitor cells have a more limited capacity for self-renewal and represent an "intermediate" between stem cells and terminally differentiated ones. Endothelial progenitor cells (EPCs) are circulating, bone marrow–derived cells that possess the ability to differentiate and mature into endothelial cells that may repair and regenerate damaged vessels.[26,27] A large number of studies have demonstrated that systemic administration of EPCs can treat experimentally induced lung injuries. Whether this includes structural contributions of the administered cells, paracrine stimulation of endogenous vascular progenitor cells, or other paracrine immunomodulatory actions remains unclear.[28]

In animal models of lung disease, engraftment of lung epithelium, vasculature, or interstitium by exogenously administered stem or progenitor cells (of bone marrow or other nonlung origin) is believed to occur rarely and with uncertain physiologic relevance.[20,25,29,30] The therapeutic effects of stem or progenitor cells are exerted through a combination of mechanisms, including transdifferentiation of a small subset of cells, direct cell-to-cell connections with transfer of cell components, release of angiogenic, antiapoptotic, and anti-inflammatory factors, and release of other cellular components, such as microvesicles or exosomes.[25]

PAH-INDUCED RVF AND INTERSTITIAL MYOCARDIAL FIBROSIS

Pressure overload–induced RVH is associated with differentiation of fibroblasts to myofibroblasts that proliferate rapidly and secrete large quantities of collagen.[31] The normally prevalent collagen III, which confers elastic properties, is replaced by collagen I that is inelastic, thereby impairing diastolic relaxation.[32] In rats with RVF, by ≈4 weeks after monocrotaline (MCT) injection (60 mg/kg), RV fibrosis had occurred in the epicardial layers (17% ± 2% vs. 1.5% ± 0.4%; $P < 0.01$) and in the endocardial layers (18% ± 3% vs. 1.4% ± 0.3%; $P < 0.01$). The LVs of these hearts did not develop fibrosis.[33] Yen et al.[34] found that 3 weeks after MCT injection, the rats suffered from RV fibrosis, had high expression of fibrotic biomarkers (transforming growth factor β [TGFβ], p-SMAD3) in the RV, and low expression of antifibrotic biomarkers (bone morphogenetic protein 2 [BMP2], p-SMAD1/5). Treatment with a combination of sildenafil and bone marrow–derived EPCs was able to reverse these changes to levels almost equal to those of healthy rats, and this combination was more potent than either of the two therapies alone.[34] Hessel et al.[35] demonstrated that in the RV of MCT-treated (80 mg/kg) hearts, at 4 weeks after MCT

injection, tenascin-C (TNC) was expressed at the messenger RNA level and at the protein level (from 0.0 to 0.62 ± 0.64 ng/mg). In the heart, TNC is an extracellular matrix glycoprotein that is expressed during cardiogenesis but disappears in the myocardium after birth. TNC may reappear in the heart under various pathological conditions, including acute myocardial infarction (MI), myocarditis, and dilated cardiomyopathy.[36] In rats with MCT-induced RVF, Umar et al.[37] demonstrated that the high TNC levels in RV myocardium at 4 weeks after MCT injection were almost absent if treated with MSCs at day 14. These studies demonstrate that PAH is associated with pathological RVH and interstitial RV fibrosis that both reverse upon cell therapy with stem and progenitor cells.

PAH-INDUCED RVF AND ARRHYTHMOGENESIS

At ≈4 weeks after MCT injection, cardiomyocytes isolated from hypertrophied RVs had prolonged their action potential duration (APD_{90}) from 33 ± 6 ms (control) to 57 ± 8 ms ($P < 0.001$). Cardiomyocytes isolated from the LVs of these hearts did not show action potential prolongation. Cardiomyocytes isolated from the RVs of MCT-treated hearts manifested greater dispersion in APD_{90} than did those from controls: 30 ± 1.8 ms vs. 20 ± 1.5 ms ($P < 0.01$).[33] The reduced repolarization reserve in the MCT-treated hearts was associated with downregulation of at least 2 K^+ channels, Kv1.5 (protein expression reduced by 88%) and KCNE2 (protein expression reduced by ≈75%). In combination with an increased susceptibility to depolarization-induced repetitive activity, all conditions for precipitation of sudden death are present in cardiomyocytes isolated from RVs of MCT-treated hearts. Sudden death occurred ≈1 week after the drop in RV ejection fraction.[33] As to cell therapy for RVF in animal models of PAH, a reduction of mortality has been reported in numerous studies,[38-43] but the exact mechanisms by which cell therapy repaired the pathologic changes associated with sudden death are not yet known.

Cardiomyocytes are electrically coupled via gap junctions and are built up of several proteins, including connexins, mainly connexin-43 (Cx43). Hearts that fail because of pressure overload have diminished Cx43 density at the intercalated disks,[44,45] which slows down conduction velocity through the myocardium. In RVs of rats with MCT-induced PAH, Cx43 staining decreased at the intercalated disks and intensified at the lateral cell membrane.[46,47] After 3 weeks of treatment with bosentan, a dual endothelin receptor antagonist, the Cx43 staining had redistributed to the intercalated disks, as in controls.[47] In a prevention study, administration of bone marrow–derived EPCs, combined with pharmacotherapy using cilostazol, a phosphodiesterase III inhibitor, to rats treated on day 3 after MCT administration was able to prevent the decrease in Cx43 expression observed in RVs of rats with MCT-induced PAH.[48] However, as the development of PAH and RVH was also prevented by this therapy, a direct effect of therapy on RV myocardial Cx43 expression is unlikely.

PAH-INDUCED RVF AND CARDIOMYOCYTE APOPTOSIS

An increased rate of cardiomyocyte apoptosis has been detected in failing human hearts,[49,50] as well as in hearts of animals with experimentally induced hypertrophy and cardiomyopathy[16] and in RVs of rats with SU5416 (a VEGF-receptor-2 antagonist)-induced and chronic hypoxia-induced PAH.[51] Stem cells from human amniotic fluid (hAFS) or cells from rat adipose tissue stromal vascular fraction (rSVC), administered intravenously in rats with MCT-induced PAH, engrafted in the lungs, heart, and skeletal muscle and differentiated into endothelial cells and vascular smooth muscle cells (VSMCs).[52] Cell therapy for PAH with hAFS or rSVC was successful in treating PAH and was associated with reduction in the number of apoptotic cells per cubic millimeter in lung, heart, and skeletal muscle toward levels found in corresponding organs of healthy rats.[52] Successful therapy for PAH is likely to include antiapoptotic actions.

PAH-INDUCED RVF: REVERSIBLE OR NOT?

The structural and functional changes associated with the state of RVF are potentially reversible, given the many reports about successful therapy for MCT-induced PAH, hypoxia-induced PAH, and high flow–induced (by shunting) PAH in experimental animals, including (1) a decrease in pulmonary vascular resistance, (2) a decrease in pulmonary artery pressure, (3) a decrease in RV mass, and (4) an increase in RV ejection fraction. Other convincing evidence of the reversible nature of PAH-induced RVF comes from studies that have addressed RV function after lung transplantation for end-stage PAH[53-55] and after successful pulmonary endarterectomy in patients with chronic thromboembolic PH.[56] Following these surgical interventions, RV function slowly recovered to normal, and this recovery was sustained in long-term follow-up. Improvements included a return of the leftward-bowing interventricular septum (IVS) to its physiological position and a disappearance of high-grade tricuspid insufficiency.[55,56] Whether there is a point of no return is to be seen, but so far numerous data on therapies for PAH, including cell therapies, indicate re-

versibility of PAH-induced RVF, albeit slow and maybe incomplete.

ENGRAFTMENT OF BONE MARROW STEM OR PROGENITOR CELLS IN THE HEART

To investigate whether circulating bone marrow–derived cells are capable of de novo cardiomyocyte formation, Deb et al.[57] analyzed hearts of 4 female patients who had undergone sex-mismatched bone marrow transplantation. The time interval between transplantation and death was 35, 480, 510, and 600 days. The mean percentage of Y chromosome–positive cardiomyocytes was 0.23% ± 0.06%, without evidence that these chimeric cardiomyocytes were the result of cell fusion.[57] Thiele et al.[58] found higher numbers of chimeric cardiomyocytes in the heart after bone marrow–derived (stem) cell transplantation. In hearts of 7 patients who received sex-mismatched bone marrow cells, 5.4% ± 1.1% of the cardiomyocytes were sex mismatched, and in the coronary vessels 5.5% ± 2.2% of the endothelial cells and 3.8% ± 0.6% of the VSMCs were sex mismatched. Four other patients received sex-mismatched peripheral blood stem cells that contained up to 2% $CD34^+$ progenitor cells. In this group 1.7% ± 0.5% of the cardiomyocytes were sex mismatched, and in the coronary vessels 4.6% ± 1.1% of the endothelial cells and 3.7% ± 0.5% of the VSMCs were sex mismatched.[58] Spees et al.[59] investigated the effects of MCT on homing, engraftment, and differentiation of bone marrow–derived cells in lungs and heart. To that purpose, these authors transplanted bone marrow from green fluorescent protein (GFP)-transgenic male rats into irradiated GFP-negative female rats. Three weeks after MCT injection into chimeric rats, the GFP-positive bone marrow–derived cells were found engrafted in the lungs and being differentiated into interstitial fibroblasts and pulmonary epithelial cells (Clara cells), vascular endothelial cells, and VSMCs. At the same time, the GFP-positive bone marrow–derived cells were found engrafted in the hypertrophied hearts of MCT-injected rats, with significantly more GFP-positive cells in the RV than in the LV (1.5 : 1), whereas in the control chimeric rats (no MCT), the LV contained more male DNA than the RV (1 : 0.5). In the RVs of rats with PAH, GFP-positive cells were mainly vascular cells and cardiomyocytes. Only a few GFP-positive cardiomyocytes appeared as binucleated cardiomyocytes. None of the GFP-positive vascular cells in the RV were fused cells.[59] Transplantation of EPCs in the lungs of dogs with dehydroMCT-induced PAH by injection into the lung parenchyma decreased the pulmonary artery pressure and pulmonary vascular resistance and increased cardiac output.[60]

In a prevention study, bone marrow–derived EPCs, combined with cilostazol, a phosphodiesterase III inhibitor, were administered intravenously to rats on day 3 after MCT administration.[48] Combination therapy (cells + drug) prevented (1) the development of PAH and RVH, (2) the decrease in pulmonary expression of Bcl-2 and endothelial nitric oxide synthase (eNOS), (3) the increase in pulmonary expression of caspase-3, matrix metalloproteinase 9 (MMP9), and tumor necrosis factor α (TNFα), (4) the decrease in alveolar sacs, (5) the decrease in pulmonary arterioles, and (6) the decrease in RV expression of Cx43. Combination therapy appeared superior to either EPCs or cilostazol alone in preventing MCT-induced PAH, including prevention of apoptotic and inflammatory actions in the lung and prevention of arrhythmogenesis in the RV.[48]

In PAH, the pulmonary resistance arteries are remodeled as a result of proliferation of endothelial cells and VSMCs. This vascular remodeling is considered to stimulate homing of MSCs. Takemiya et al.[42] demonstrated that more intravenously administered MSCs engrafted in lungs of rats with MCT-induced PAH at days 1 and 14 after cell administration than in lungs of healthy rats, by 2.5- and 6.5-fold, respectively. Cell therapy was given 2 weeks after MCT injection. Two weeks after cell administration, the MSCs, transduced with GFP, had not lowered the pulmonary artery pressure or the RV/(LV + IVS) weight ratio, compared to those in rats with PAH 4 weeks after MCT injection. However, if the MSCs had been transduced with prostacyclin synthase, pulmonary artery pressure and the RV/(LV + IVS) weight ratio were significantly lower than corresponding measurements in rats with PAH 4 weeks after MCT injection.[42]

Bone marrow–derived MSCs genetically modified with HGF administered intravenously 3 weeks after MCT injection in rats caused lower pulmonary artery systolic pressure, lower RV weight, less interstitial fibrosis in the RV myocardium, and increased staining of RV myocardial Cx43 at the intercalated disks, compared with rats with MCT-induced PAH without cell therapy.[61] The therapeutic effects of MSCs without genetic modification were less pronounced.

When administered intravenously in rats with MCT-induced PAH, hAFS and hSVC engrafted in the lungs (2%–4.5%), heart (0.4%–0.8%), and skeletal muscle (0.2%–0.4%) and differentiated into endothelial cells and VSMCs.[52] These stem cells caused a decrease in pulmonary artery systolic pressure, RV weight, RV dilatation, plasma concentration of brain natriuretic peptide (BNP), and numbers of apoptotic cells in lung, heart, and skeletal muscle.[52]

These studies demonstrate that cell therapy with bone marrow–derived stem and progenitor cells, administered to treat PAH, may lead to engraftment of a small fraction of stem or progenitor cells in the lungs and an even smaller fraction in the RV myocardium, with subsequent differentiation into vascular and cardiac cell lineages, respectively.

Are the beneficial effects of cell therapy on RV structure and function indirect (secondary to a decrease in pulmonary vascular resistance) or direct, e.g., due to cells that had engrafted in the heart or to cells that had engrafted elsewhere? Human MSCs that were entrapped in the lungs of rats with MI had a therapeutic effect on the heart, as evidenced by production of anti-inflammatory substances, such as TNF-inducible gene-6 protein (TSG-6), that reduced inflammatory responses and even reduced infarct size.[62] As a disease, PAH differs from MI, but a failing RV of an animal or individual with PAH may have become ischemic (or otherwise vulnerable) and may become a target for circulating cells administered as cell therapy. The so-called paracrine effects of engrafted cells on other cells, e.g., in lungs and/or heart, may be supplied by (1) soluble factors secreted by engrafted cells, as was shown by experiments in which the cells were replaced by conditioned medium,[63,64] and/or (2) exosomes, being secreted membrane microvesicles that have been isolated from conditioned media of MSCs.[65]

An example that stem cells may be able to repair "myocardial injury" is presented by Gopinath et al.[66] Human umbilical cord blood (hUCB)–derived stem cells were administered intravenously in immunoincompetent mice 14 days after the mice had been injected with doxorubicin, leading to pathological heart hypertrophy. The hUCB-derived stem cells migrated and integrated into the hearts and reversed the expression of pathological hypertrophic markers, such as ANP, β-MHC, and α-skeletal actin, whereas the expression of physiological hypertrophic markers, such as sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA2), insulin-like growth factor 1 (IGF-1), and α-MHC, increased, indicating that hUCB-derived stem cells caused a shift from pathological hypertrophy toward physiological hypertrophy in doxorubicin-challenged mice.[66] Earlier, this shift from pathological to physiological hypertrophy was noticed in mice with angiotensin II–induced LVH that were treated with hUCB-derived stem cells.[67] The hUCB-derived stem cell treatment in mice decreased the doxorubicin-induced increase in heart weight–to–body mass ratio, myocardial fibrosis, and number of apoptotic cardiomyocytes.[66] These findings suggest that cell therapy with hUCB-derived stem cells is able to reverse heart failure, but whether this applies to doxorubicin-induced heart failure only is uncertain.

CONCLUSIONS

The conditions by which a state of "compensated" RVH switches to a state of RVF are partially known: (1) development of fibrosis, (2) "fetal" gene expression, (3) apoptosis, and (4) slowed conduction due to gap junction loss may determine whether the RV starts failing. We believe that these changes are potentially reversible, given (1) the many reports about successful therapy for MCT-induced PAH, hypoxia-induced PAH, and high flow–induced (by shunting) PAH in experimental animals and (2) the reports about reversed RV remodeling after lung transplantation in patients with end-stage PAH. The therapeutic effects of stem and progenitor cell therapy are considered to be (1) paracrine effects from stem and progenitor cells that had engrafted in the myocardium (or elsewhere), by compounds that have anti-inflammatory, antiapoptotic, and proangiogenic actions and (2) unloading effects on the RV due to stem or progenitor cell–induced decrease in pulmonary vascular resistance and decrease in pulmonary artery pressure. The therapeutic effects of stem and progenitor cell therapy are improved by genetic modification of the cells with genes encoding anti-inflammatory, antiapoptotic, and proangiogenic actions.

Source of Support: Nil.

Conflict of Interest: None declared.

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