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Sildenafil improves long-term effect of endothelial progenitor cell-based treatment for monocrotaline-induced rat pulmonary arterial hypertension

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Abstract

Background aims. We hypothesized that the long-term therapeutic effect of combined sildenafil and bone marrow-derived endothelial progenitor cells (BMDEPCs) on monocrotaline (MCT)-induced rat pulmonary arterial hypertension (PAH) is superior to either treatment alone. *Methods.* Male Sprague-Dawley rats ($n = 40$) were equally divided into normal controls, MCT (65 mg/kg, subcutaneously) only, MCT + sildenafil (25 mg/kg/day, orally), MCT + BMDEPCs (2.0×10^6 autologous cells, intravenously) and MCT + sildenafil+ BMDEPCs. BMDEPCs and sildenafil were given on day 21 after MCT administration. Animals were sacrificed by day 90 after MCT administration. *Results.* The apoptotic (caspase 3, Bax) and inflammatory (tumor necrosis factor-α, matrix metalloproteinase-9) biomarkers in right ventricle and lung and pulmonary expressions of fibrotic biomarkers (transforming growth factor-β, p-Smad3) and connexin 43 protein were lower in monotherapy groups (i.e., MCT + sildenafil and MCT + BMDEPCs) and further decreased in normal controls and combined treatment groups (i.e., MCT + sildenafil + BMDEPCs) compared with untreated animals (i.e., MCT only) (all $P < 0.01$). Expressions of anti-fibrotic biomarkers (bone morphogenetic protein-2, p-Smad1/5) and numbers of alveolar sacs and arterioles in lung were higher in monotherapy groups and further increased in normal controls and combined treatment groups compared with untreated animals (all $P < 0.005$). In right ventricle, connexin 43 and α-myosin heavy chain (MHC) expressions were higher in the monotherapy groups and further elevated in normal controls and combined treatment groups compared with untreated animals, whereas β-MHC exhibited the opposite pattern (all $P < 0.01$). Right ventricular systolic pressure and weight were lower in the monotherapy animals and further reduced in normal controls and combined treatment groups compared with untreated animals (all $P < 0.0001$). *Conclusions.* Combined therapy with BMDEPCs and sildenafil was superior to either treatment alone in attenuating rodent MCT-induced PAH.

Key Words: *bone marrow-derived endothelial progenitor cells, combined therapy, MCT-induced pulmonary hypertension, sildenafil*

Introduction

Abundant data have established that pulmonary arterial hypertension (PAH) is attributable to a progressive increase in pulmonary vascular resistance and vascular wall remodeling caused by vascular cell proliferation and obliteration of pulmonary microvasculature, leading to severe PAH and right-sided heart failure (1–4). Severe PAH can drastically limit physical capacity and reduce the median life expectancy to only 2.8 years following diagnosis without treatment (5,6).

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Based on the pathogenesis of PAH, three different classes of medications, prostacyclins, endothelin-1 receptor antagonists and phosphodiesterase5 inhibitors, have become the mainstays of treatment in the last decade (7). Although it has been well established that these medications are effective in hindering the progression of PAH (7–10), the disease remains incurable. A novel effective therapeutic approach is desirable.

Although the pathogenesis of PAH is multifactorial, dysfunction of endothelial cells in pulmonary arteries has been identified to play the most crucial role in the initiation and development of PAH (11–14). This dysfunction ultimately causes an imbalance between injury of endothelial cells and the capacity for repair of damaged endothelial cells in pulmonary arteries (1,11,12). Previous studies have shown that circulating endothelial progenitor cells (EPCs) play a role in the endothelial repair process and neovascularization (15–17). Based on these findings (15–17) and the pathogenesis of PAH (1,11,12), cell-based therapy using EPCs has been applied to the treatment of PAH in animal models and some clinical studies (18–22). Although these studies have demonstrated promising results of short-term EPC treatment (19–22), the long-term effect remains uncertain. Our previous study (21) showed that long-term use of EPC monotherapy for monocrotaline (MCT)-induced rat PAH was partially effective in preserving normal pulmonary hemodynamics. Our previous investigation also demonstrated that sildenafil, a phosphodiesterase 5 inhibitor, significantly alleviated MCT-induced PAH through suppressing pulmonary vascular remodeling (23). However, when alleviated pulmonary arterial remodeling (i.e., histopathologic findings) and PAH (i.e., functional evaluation) were taken as therapeutic endpoints, the therapeutic efficacy of this drug remained unsatisfactory (23).

Our previous study revealed that sildenafil therapy enhanced endothelial nitric oxide synthase (eNOS) production and basal nitric oxide release, indexes of endothelial protection, repair, proliferation and angiogenesis (23). Based on these previous reports (11–14,19–22), it is rational to hypothesize that sildenafil-assisted cell-based therapy may provide an additional benefit in improving PAH. The hypothesis is of clinical interest because sildenafil is a phosphodiesterase inhibitor approved by the U.S. Food and Drug Administration for clinical use in PAH and because stem cell therapy has already been applied to clinical practice for various ischemia-related organ diseases. Combined use of these two therapeutic options in patients with PAH is feasible without ethical debate. Using a rat model, the present study was designed to test the hypothesis that combined treatment with sildenafil and EPCs could provide additional long-term benefit in ameliorating MCT-induced PAH.

Methods

Ethics

All animal experimental procedures were approved by the Institute of Animal Care and Use Committee at our hospital and performed in accordance with the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1996).

Animal models of PAH

On day 0, 32 pathogen-free, adult male Sprague-Dawley rats, weighing 300–320 g (BioLASCO Co, Ltd, Taipei, Taiwan), were given one subcutaneous injection of MCT (65 mg/kg; Sigma, St Louis, MO, USA). The MCT-treated animals were assigned to MCT only ($n = 8$), MCT + sildenafil ($n = 8$), MCT + bone marrow-derived endothelial progenitor cells (BMDEPCs) ($n = 8$) and MCT + sildenafil + BMDEPC ($n = 8$) groups. The dosages of the drugs (i.e., MCT and sildenafil) were based on our previous studies with some modifications (21, 23, 24). Another group of eight Sprague-Dawley rats (i.e., normal controls) received only subcutaneous injection of 3 mL of normal saline. Sildenafil (25 mg/kg/day, orally) and BMDEPCs ($2.0–2.2 \times 10^6$ given intravenously once via the penile vein) were administered on day 21 after MCT injection.

Rationales of choosing treatment on day 21 after MCT-induced PAH and therapeutic period of 90 days

This was the third year study supported by a Program Grant from the National Science Council, Taiwan (grant NSC-97-2314-B-182A-022-MY3). The rationales of the current experimental design were based on two considerations. First, because clinical PAH is frequently diagnosed at a late stage of the disease, it was important to elucidate the timing when MCT-induced PAH has adequately developed. In the current study, an additional five adult male Sprague-Dawley rats were treated with MCT (65 mg/kg) to serve this purpose. Based on our previous experience (21,23), the mean right ventricular systolic pressure (RVSP) of Sprague-Dawley rats at day 21 after MCT treatment was 40.4 ± 3.3 mm Hg, indicating a significant PAH. The initial treatment timing was based on the results of the pilot study. Second, to investigate whether this combined regimen is potentially effective in the clinical treatment of PAH, we attempted to elucidate