Sequential and timely transfection of hepatocyte growth factor and monocyte chemotactic protein-1 ameliorates hyperkinetic pulmonary artery hypertension in rabbits

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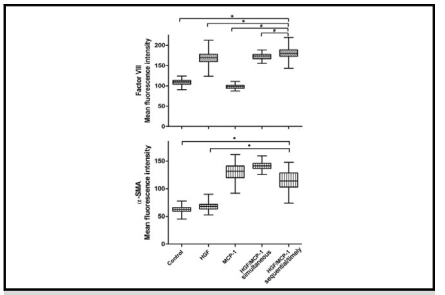
ABSTRACT

Objective: To investigate the effect of sequential and timely transfection of the recombinant human hepatocyte growth factor (hHGF) gene and human monocyte chemotactic protein-1 (hMCP-1) gene on hyperkinetic pulmonary artery hypertension in a rabbit model.

Methods: The rabbits with pulmonary artery hypertension were randomly separated into 5 groups: control; hHGF; hMCP-1; hHGF/hMCP-1 simultaneous transfection; and hHGF/hMCP-1 sequential, timely transfection. Two weeks after the transfection, real-time polymerase chain reaction and immunohistochemistry examination were used to detect the expression of hHGF and hMCP-1. Four weeks later, the hemodynamic parameters were measured, and immunohistochemical and immunofluorescence staining were performed, to investigate microvascular density and arterialization.

Results: The final adenovirus coding with enhanced green fluorescent protein-hMCP-1 virus was 3×10^{10} plaque-forming units/mL, and the purity of adenovirus coding with hHGF was 1.31. Three days after the transfection, enhance green fluorescent protein hMCP-1 green fluorescence was detected in the lung tissues and increased to its peak point in 1 week. Two weeks later, hHGF and hMCP-1 were expressed in all transfection groups. By the end of 4 weeks, the mean pulmonary artery pressure in the hHGF/hMCP-1 sequential and timely transfection group was lower than that in the other groups. Confirmed by immunohistochemical and immunofluorescence staining, the microvascular and arteriolar density in the lung tissues of the sequential and timely hHGF/hMCP-1 transfection group were higher than that in the other groups.

Conclusions: Expression of hHGF and hMCP-1 were found in rabbit lung after gene transfection via an airway approach. By increasing the pulmonary microvascular density and promoting arterializations, sequential and timely hHGF/hMCP-1 transfection ameliorates the shunt flow–induced pulmonary artery hypertension. (J Thorac Cardiovasc Surg 2015;150:634-43)



Results of factor VIII (microvascular density) and α-SMA (arteriole distribution) staining of lung tissues. * and #, $P < .05$, 1-way ANOVA with Fisher-Hayter least significant difference procedure.

Central Message

Expression of hHGF and hMCP-1 were found in rabbit lungs after gene transfection via an airway approach. By increasing the pulmonary microvascular density and promoting arterializations, sequential and timely hHGF/hMCP-1 transfection ameliorates shunt flow–induced pulmonary artery hypertension.

Perspective

Hyperkinetic PAH caused by a left-to-right shunt in congenital heart disease is characterized by a decrease of the available pulmonary vascular bed. Development of new therapeutic strategies is critical, to improve the prognosis of this devastating disease. Gene-based angiogenesis therapy is an area of increasing interest to researchers hoping to reduce vascular resistance and improve tissue perfusion. However, the puzzle of how to transform immature neovascular vessels into functional arterioles remains unsolved. We demonstrated that sequential and timely transfection of HGF/MCP-1 ameliorates PAH in rabbits by promoting the growth and arterialization of neovascular microvessels, a new approach to treating PAH.

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Abbreviations and Acronyms

Ad = adenovirus
EGFP = enhanced green fluorescent protein
hHGF = human hepatocyte growth factor
HGF = hepatocyte growth factor
hMCP-1 = human monocyte chemotactic protein-1
MCP-1 = monocyte chemotactic protein-1
mRNA = messenger ribonucleic acid
PAH = pulmonary arterial hypertension
Pfu = plaque-forming units
SMA = smooth muscle actin
SMC = smooth muscle cell
VEC = vascular endothelial cell

Supplemental material is available online.

Hyperkinetic pulmonary artery hypertension (PAH) is a common and problematic complication of congenital heart disease with left-to-right shunt. This condition is associated with increased mortality and therefore seriously affects the prognosis of such patients.[1] Previous studies suggested that acinar pulmonary arteries are key regulators of pulmonary hemodynamics and luminal stenosis, owing to the fact that thickening pulmonary arterial walls contribute to PAH.[2]

Currently, vascular dilatation, via inhalation of nitrous oxide, with use of nitric acid lipid drugs and prostacyclin, is recommended as a first approach to treating the disease.[3,4] Although palliation of PAH can be achieved with surgical manipulation,[5,6] and pharmacotherapies can prolong the survival of patients with PAH,[7] the long-term outcome is not favorable because of the obstructive remodeling of pulmonary arterioles and the decrease in the available pulmonary vascular bed.[8] In recent years, gene-based angiogenesis therapy has become an area of increasing interest in many research fields. Therapeutic angiogenesis reduces vascular resistance and improves perfusion of tissues.[9,10]

Hepatocyte growth factor (HGF) is a multifunctional growth factor evolving from mesenchymal origin, bound to its specific membrane receptor, c-met, to induce diverse physiologic activities.[11] In recent years, several studies have shown that HGF can promote endothelial cell mitosis more effectively than vascular endothelial growth factor, without increasing vascular permeability.[12,13] Furthermore, it can suppress vascular endothelial cell (VEC) apoptosis, and protect neovascularization from degradation.[11] Our previous study suggested that instilling Ad-hHGF could effectively transfect alveolar epithelium, bronchial epithelium, and pulmonary VECs, and allow the microvessels in the lung tissues to fluorish. But most of them were only nonmuscular, immature microvasculature, limiting their function to reducing pulmonary artery pressure.[14]

The method of transforming neovascular vessels into arterioles is critical to effective reduction of pulmonary artery pressure. Monocyte chemotactic protein-1 (MCP-1), usually secreted by VECs, smooth muscle cells (SMCs), or macrophages, is a chemotactic factor belonging to the chemotactic cytokine family. It can promote the mitosis of vascular endothelium, adhere to CCR2 receptor, and evoke oriented chemotaxis of VECs.[15] Furthermore, MCP-1 can promote the mitosis of VECs, and urge VECs and mesenchymal cells to move toward VECs, thereby accelerating the process of neovascular arterialization.[16]

In this study, hHGF was transfected in the lungs of PAH rabbit models to induce the development of pulmonary microvessels. Afterward, hMCP-1 was sequentially transfected in a timely fashion to promote the growth and arterialization of neovascular microvessels. The collateral circulation with physiologic function was constructed to decrease the impedance of the pulmonary bloodstream and the pulmonary blood pressure. The experimental study was designed to determine whether sequentially and timely transfected HGF/MCP-1 can be used in treating pulmonary hypertension, and its effectiveness.

METHODS

Animal Models

The study was conducted in accordance with the Guide for the Care and Use of Laboratory Animals of the National Research Council of China. The experiment was approved by the Animal Care Committee of the Xuzhou Medical College of China. The animals received humane care in compliance with the Guide for the Care and Use of Laboratory Animals of Jiangsu Province of China. One-month-old New Zealand male rabbits (weighing 540 ± 30 g) were supplied by the Laboratory Animal Center of Xuzhou Medical College. A systemic-pulmonary shunt was established with left common carotid artery and pulmonary trunk anastomosis, as described previously.[17] The pulmonary hypertension model was established after 3 months. A total of 110 rabbits were utilized, and 77 rabbits survived after 3 months, a survival rate of 70%. In addition, 12 healthy rabbits were used for detecting hMCP-1 expression in lung tissue.

Detection of hMCP-1 Expression

Twelve normal rabbits were randomly separated into 4 groups, and endotracheally instilled with Ad-enhanced green fluorescent protein (EGFP)-hMCP-1 (2×10^9 Pfu per rabbit). Enhanced green fluorescent protein–hMCP-1 fluorescence and real-time polymerase chain reaction were employed to detect expression of hMCP-1 in the lung tissues.

Preparation of Recombinant Adenoviral Vector

Recombinant adenovirus (replication-defective human serum adenovirus type 5 with cytomegalovirus promoter) coding with human HGF (Ad-hHGF) were constructed by the Academy of Military Medical

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