Novel approach with intratracheal administration of microgelatin hydrogel microspheres incorporating basic fibroblast growth factor for rescue of rats with monocrotaline-induced pulmonary hypertension

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Objectives: Pulmonary hypertension is a life-threatening disease, and alternative strategies are essential for patients with critical pulmonary hypertension. We developed a new procedure using microgelatin hydrogel microspheres incorporating basic fibroblast growth factor (mGHMs/bFGF) for intratracheal administration and evaluated the effect of a single intratracheal administration of mGHMs/bFGF on rats with monocrotaline-induced pulmonary hypertension.

Methods: Monocrotaline was injected into 54 rats simultaneously with intratracheal administration of plain mGHMs (vehicle group), bFGF in solution form (free-bFGF group), mGHMs/bFGF (mGHMs/bFGF group), and plain saline with subcutaneous injection of saline instead of monocrotaline (control group, n = 18). Three weeks after the administration, 48 rats (n = 12 from each group) were subjected to hemodynamic and histologic evaluations. Survival was assessed in 6 rats of each group 10 weeks after the intratracheal administration.

Results: The mGHMs/bFGF group showed significantly lower right ventricular/left ventricular pressure ratios at 3 weeks than the vehicle and free-bFGF groups (0.35 ± 0.04, 0.54 ± 0.11, 0.58 ± 0.21, and 0.36 ± 0.05 for the control, vehicle, free-bFGF, and mGHMs/bFGF groups, respectively; $P < .01$). Histologically, the mGHMs/bFGF group had a significantly higher number of vessels (diameter ≥50 μm) than the other groups (5.3 ± 2.6, 4.6 ± 2.8, 7.3 ± 2.5, and 18.9 ± 7.0 vessels/mm^2, respectively; $P < .01$). Ten weeks after the intratracheal administration, 6 (100.0%) rats had survived in the control group, and 1 (16.7%) survived in the vehicle, 0 (0%) in the free-bFGF, and 5 (83.3%) in the mGHMs/bFGF groups (n = 6 each).

Conclusions: A single intratracheal administration of mGHMs/bFGF increased the number of vessels in the lung and ameliorated survival and hemodynamics in rats with monocrotaline-induced pulmonary hypertension.

CSP

Pulmonary hypertension (PH) is a life-threatening disease characterized by progressive pulmonary arterial hypertension and increases in pulmonary vascular resistance that lead to right ventricular (RV) failure.[1] Recently, survival and quality of life have improved in patients with PH because of the development of pharmacologic therapies, such as novel types of prostacyclin,[2,3] endothelin receptor blockers,[4,5] and phosphodiesterase inhibitors.[6,7] However, these pharmacologic effects might be transient or not sufficient for the severely damaged pulmonary vessels, such as critical PH. Thus alternative strategies are essential for patients with critical PH.

Basic fibroblast growth factor (bFGF) is not only a potent angiogenic mitogen of various kinds of cells but also a cytokine that can stimulate tissue regeneration.[8-10] We developed a biodegradable hydrogel made from acidic gelatin as the slow-release carrier for bFGF and demonstrated that sustained release of bFGF with biodegradable gelatin hydrogels enhanced angiogenesis and arteriogenesis in various cardiovascular areas.[11-14] Although the angiogenic and arteriogenic properties of bFGF can ameliorate severe PH, the optimal delivery method to the lung has not been established. In the study presented here, we used newly developed microgelatin hydrogel microspheres (mGHMs) that make intratracheal administration possible.

We used rats with monocrotaline (MCT)-induced PH to test the hypothesis that a single intratracheal administration of mGHMs incorporating bFGF (mGHMs/bFGF) could enhance both angiogenesis and arteriogenesis in the lung and ameliorate progressive PH.

MATERIALS AND METHODS

Preparation of mGHMs/bFGF

Gelatin with an isoelectric point of 4.9 was isolated from bovine bone collagen by using an alkaline process with $Ca(OH)_2$ (Nitta Gelatin Co, Osaka, Japan). Human recombinant bFGF with an isoelectric point of 9.6

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Abbreviations and Acronyms

bFGF	= basic fibroblast growth factor
LV	= left ventricle
MCT	= monocrotaline
mGHM	= microgelatin hydrogel microsphere
mGHMs/bFGF	= microgelatin hydrogel microspheres incorporating basic fibroblast growth factor
PH	= pulmonary hypertension
RV	= right ventricle

was purchased from Kaken Pharmaceutical Co, Ltd (Tokyo, Japan). mGHMs with a diameter of approximately 10 μm were made as previously described[15] and impregnated with an aqueous solution containing 100 μg of bFGF and mGHMs/bFGF. All experimental procedures were conducted under sterile conditions.

Animals

Male Wistar rats weighing 200 to 220 g were used in this study. All animals received humane care in compliance with the ''Principles of laboratory animal care'' formulated by the National Society for Medical Research and the ''Guide for the care and use of laboratory animals'' prepared by the Institute of Laboratory Animal Resources, National Research Council, and published by the National Academy Press.

Experimental PH Model

MCT (300 mg, Crotaline; Sigma, St Louis, Mo) was dissolved in 1.8 mL of 1 mol/L HCl, followed by the addition of 3 to 4 mL of distilled water.[16] This solution was then adjusted to pH 7.4 with 1 mol/L NaOH solution and topped up to 15 mL with distilled water. Each rat was anesthetized with an intraperitoneal injection of sodium pentobarbital (30 mg/kg) and received a single subcutaneous injection of MCT solution (60 mg/kg) in the neck. After this injection, PH gradually progressed and was completed approximately 3 weeks after the injection,[16] whereas lung tissues displayed interstitial thickening accompanied by prominent medial hypertrophy of the muscular pulmonary artery and arterioles.[17]

Release Profile of bFGF From mGHMs in Lung Tissue After Intratracheal Administration

The intrapulmonary release profile of bFGF from mGHMs after intratracheal administration was evaluated in 30 healthy rats. The rats were anesthetized as described above, and mGHMs incorporating iodine 125–labeled bFGF (100 μg/0.1 mL) were injected into the trachea of 15 rats with a pulmonary drug delivery device (Micro Sprayer IA-1B; LMS Co, Ltd, Tokyo, Japan; mGHMs/bFGF group, n = 15). In the other 15 rats, iodine 125–labeled bFGF solution (100 μg/0.1 mL) was injected into the trachea with the same device (free-bFGF group, n = 15). Bilateral pulmonary tissues were obtained 2 hours and 3, 7, 10, and 14 days after the intratracheal administration (n = 3 for each time point). The radioactivity remaining in each lung was measured with a gamma counter (ARC-301B; Aloka, Tokyo, Japan).

Prevention of Progression of PH by mGHMs/bFGF

Just after the MCT injection, 54 rats were randomized to one of 3 groups (n = 18 each) and underwent intratracheal administration of the following solutions: 0.1 mL of saline containing free mGHMs (vehicle group), 0.1 mL of saline containing 100 μg of bFGF (free-bFGF group), and 0.1 mL of saline containing mGHMs incorporating 100 μg of bFGF (mGHMs/bFGF group). The remaining 18 rats received a single subcutaneous injection of saline instead of MCT, followed by intratracheal administration of 0.1 mL of saline (control group). Three weeks after the administration, 12 rats from each group were subjected to hemodynamic and histologic evaluations. Survival was assessed in 6 rats of each group 10 weeks after the intratracheal administration.

Measurement of Hemodynamic Parameters and Assessment of RV Hypertrophy

Three weeks after the intratracheal administration, rats were anesthetized and mechanically ventilated (fraction of inspired oxygen, 0.21; respiratory rate, 60 breaths/min). After the heart was exposed after median sternotomy, systolic pressures of the RV and left ventricle (LV) were measured by means of puncture with a 23-gauge needle. The rats were then killed by means of intravenous administration of a lethal dose of sodium pentobarbital, and the heart and bilateral lungs were thoroughly washed with saline and sampled en bloc under pressurization through an intubation catheter. To assess RV hypertrophy, the RV free wall was dissected from the LV and septum, and they were weighed separately with an analytic scale. Lungs were fixed with 10% paraformaldehyde solution in PBS solution for histologic examination.

Arterial Blood Gas Analysis

In conjunction with the hemodynamic studies, an arterial blood sample of approximately 0.2 mL was obtained from the needle inserted into the LV and subjected to blood gas analysis.

Histopathology

For the production of paraffin sections, the lung tissue was fixed in formalin for 24 hours at room temperature, dehydrated in ethanol, and embedded in a paraffin block. Sections were cut into 3-μm-thick specimens and stained with hematoxylin and eosin. Tissue sections were also immunostained with antibodies against von Willebrand factor (an endothelial marker). Normal immunoglobulin fractions were used as a negative control to determine antibody specificity. Five fields were randomly selected in each sample, and the numbers of vessels (50 μm $\leq$ diameter) and small vessels, capillaries, or both (diameter < 50 μm) per square millimeter were counted manually by 2 pathologists who were unaware of the treatment groups. Medial wall thickness was also assessed as the percentage of medial wall thickness by 2 pathologists using elastica van Gieson stain, as described previously.[18]

Statistical Analysis

All data was expressed as means $\pm$ standard deviations and as ranges. Differences between groups were assessed by means of analysis of variance, followed by post hoc comparisons with the Bonferroni/Dunn method. Significance of differences in survival data were determined by means of the Kaplan–Meier analysis and compared by using log-rank tests. Statview software (Abacus Concepts, Inc, Berkeley, Calif) was used for all statistical analyses.

RESULTS

Release Profile of bFGF From mGHMs in the Lung After Intratracheal Administration

None of the mice died after the intratracheal administration of free-bFGF or mGHMs/bFGF. bFGF was distributed equally in both the mGHMs and mGHMs/bFGF groups in terms of the volume in each lobe of the lung.(Radioactivity remaining in the right upper lobe, right middle lobe, right lower lobe, left upper lobe, and left lower lobe of total lung 2 hours after the intratracheal administration was 10.5% $\pm$ 3.9%, 11.3% $\pm$ 5.5%, 24.6% $\pm$ 8.4%, 43.8%