Gene Transfer of Human Prostacyclin Synthase Ameliorates Monocrotaline-Induced Pulmonary Hypertension in Rats

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Background—Prostacyclin is a potent vasodilator that also inhibits platelet adhesion and cell growth. We investigated whether in vivo gene transfer of human prostacyclin synthase (PGIS) ameliorates monocrotaline (MCT)-induced pulmonary hypertension in rats.

Methods and Results—The cDNA encoding PGIS was intratracheally transfected into the lungs of rats by the hemagglutinating virus of Japan–liposome method. Rats transfected with control vector lacking the PGIS gene served as controls. Three weeks after MCT injection, mean pulmonary arterial pressure and total pulmonary resistance had increased significantly; the increases were significantly attenuated in PGIS gene–transfected rats compared with controls [mean pulmonary arterial pressure, 31±1 versus 35±1 mm Hg (−12%); total pulmonary resistance, 0.087±0.01 versus 0.113±0.01 mm Hg · mL · min⁻¹ · kg⁻¹ (−23%), both P<0.05]. Systemic arterial pressure and heart rate were unaffected. Histologically, PGIS gene transfer inhibited the increase in medial wall thickness of peripheral pulmonary arteries that resulted from MCT injection. PGIS immunoreactivity was intense predominantly in the bronchial epithelium and alveolar cells. Lung tissue levels of 6-keto-PGF₁α, a stable metabolite of prostacyclin, were significantly increased for ≥1 week after transfer of PGIS gene. The Kaplan-Meier survival curves demonstrated that repeated transfer of PGIS gene every 2 weeks increased survival rate in MCT rats (log-rank test, P<0.01).


Key Words: prostaglandins ■ gene therapy ■ hypertension, pulmonary ■ viruses
amplifies monocrotaline (MCT)-induced pulmonary hypertension, and improves survival in MCT rats.

Methods

Animals
Male Wistar rats weighing 100 to 120 g were used in this study. Rats were randomly assigned to receive intratracheal injection of either the HVJ-liposome complex with PGIS expression plasmid or control vector, followed by a subcutaneous injection of either 60 mg/kg MCT or 0.9% saline. This protocol resulted in the creation of 4 groups: MCT rats transfected with PGIS expression plasmid (MCT-PGIS, n=8); MCT rats transfected with control vector (MCT-CON, n=8); normal rats transfected with PGIS expression plasmid (NL-PGIS, n=8); and normal rats transfected with control vector (NL-CON, n=8). Another 40 rats injected with MCT were studied to assess the time course of prostacyclin synthesis after transfer of either PGIS expression plasmid or control vector. An additional 24 rats were studied to evaluate the effects of PGIS gene therapy on survival in MCT rats.

Construction of Plasmid DNA
The expression vector for human PGIS was constructed as described previously. In brief, the blunt HindIII/BamHI fragment of the full-length human PGIS cDNA was ligated into the blunt XhoI site of the pUC-CAGGS expression plasmid. To verify that the pUC-PGIS construct encoded a biologically active PGIS protein, pUC-PGIS was transfected into 293 cells, and PGIS activity in the transfected cells was measured as conversion of [14C]PGH2 to 6-keto-[14C]PGF1α. The pUC-CAGGS vector lacking the insert encoding human PGIS served as the control vector.

Preparation of HVJ-Liposomes
Preparation of HVJ-anionic liposomes with a lipid composition similar to that of the viral envelope (AVE) has been described elsewhere.15 In brief, lipid mixtures were prepared by mixing lipid solutions (phosphatidylcholine/diiodophosphatidyl ethanolamine/sphingomyelin/phosphatidylserine/cholesterol, 4:4:4:3:15 in molar ratio). Dried lipid mixture was propagated with plasmid DNA (200 μg) in 200 μL balanced salt solution (mmol/L: NaCl 137, KCl 5.4, Tris-HCl 10 [pH 7.6]). HVJ-liposomes were prepared by mixing the lipid with HVJ virus inactivated by ultraviolet irradiation. After incubation and sucrose gradient centrifugation, the HVJ-liposome complex was collected with a Pasteur pipette.

Experimental Protocol
After the rats were anesthetized by intraperitoneal injection of pentobarbital (30 mg/kg), tracheotomy was performed to introduce a polyethylene catheter (PE-50) into the left and right main bronchi. Through the catheter, 400 μL HVJ-liposome complex including 100 μg plasmid DNA was administered into the bronchi. Next, rats were intubated with a polyethylene tube (PE-240) and artificially ventilated with a volume-regulated respirator for 30 minutes. Then, the polyethylene tube was removed and the tracheotomy was closed. Finally, rats were given a single subcutaneous injection of either 60 mg/kg MCT or 0.9% saline 24 hours after gene transfer. Animals were maintained on standard rat chow.

Hemodynamic studies were performed 22 days after gene transfer. In the MCT-injected rats, 24 rats received repeated administration of 200 μL HVJ-liposome complex including 50 μg plasmid DNA was done via tracheal tube (PE-240). Rats were then ventilated with a volume-regulated respirator for 30 minutes.
Survival was estimated from the date of MCT injection to the death of the rat or 10 weeks after the injection.

**Statistical Analysis**

All data were expressed as mean±SEM unless otherwise indicated. Comparisons of parameters between 2 groups were made by unpaired Student's t test. Comparisons of parameters among 4 groups were made by 1-way ANOVA, followed by Newman-Keuls test. Survival curves according to the presence or absence of PGIS gene transfer were derived by the Kaplan-Meier method and compared by log-rank tests. A value of P<0.05 was considered statistically significant.

**Results**

**Physiological Profiles**

The physiological profiles of the 4 experimental groups are summarized in the Table. Body weight and mean arterial pressure were significantly lower in MCT rats (MCT-CON and MCT-PGIS groups) than in normal rats (NL-CON and NL-PGIS groups). There was no significant difference in heart rate among the 4 groups. Both RV/BW and RV systolic pressure were significantly increased in rats injected with MCT.

**Effect of PGIS Gene Transfer on MCT-Induced Pulmonary Hypertension**

Three weeks after MCT injection, pulmonary hypertension developed in both MCT groups, but the rise in mean pulmonary arterial pressure was significantly smaller (by 12%) in the MCT-PGIS group than in the MCT-CON group (31±1 versus 35±1 mm Hg, P<0.05, Figure 1). Cardiac output was significantly higher (by 13%) in the MCT-PGIS than in the MCT-CON group (360±11 versus 320±16 mL/min·kg⁻¹, P<0.05). Cardiac output tended to be decreased in the MCT-CON compared with the NL-CON group, but no reduction in cardiac output was observed in the MCT-PGIS group. Therefore, total pulmonary resistance was significantly lower (by 23%) in the MCT-PGIS than in the MCT-CON group (0.087±0.01 versus 0.113±0.01 mm Hg·mL⁻¹·min⁻¹·kg⁻¹, P<0.05). Similarly, increases in RV systolic pressure and RV/BW were significantly attenuated in the MCT-PGIS compared with the MCT-CON group (Table). In contrast, neither systemic arterial pressure nor heart rate differed between the 2 groups. There were no significant differences in hemodynamic parameters between the NL-CON and NL-PGIS groups.

**Morphometric Analysis of Pulmonary Arteries**

Representative photomicrographs showed that the hypertrophy of the pulmonary vessel wall was attenuated in MCT-PGIS rats compared with MCT-CON rats (Figure 2). Quantitative analysis of peripheral pulmonary arteries demonstrated significant increases in percent wall thickness in both MCT groups, but these changes were significantly attenuated in the MCT-PGIS compared with the MCT-CON group (25±2% versus 31±2% in the vasculature with external diameters of 25 to 50 μm; 26±2% versus 33±3% in the 51- to 100-μm vasculature, both P<0.05, Figure 3).

![Figure 1](http://circ.ahajournals.org/)

**Figure 1.** Effects of PGIS gene transfer on mean pulmonary arterial pressure (A), cardiac output (B), and total pulmonary resistance (C). Abbreviations as in text. Data are mean±SEM. *P<0.05 vs NL-CON; †P<0.05 vs MCT-CON.
was no significant difference in either parameter between the NL-CON and NL-PGIS groups.

Immunohistochemical Analysis
PGIS immunoreactivity was intense in distal bronchial epithelium in MCT rats transfected with PGIS gene (Figure 4). Diffuse immunostaining for PGIS was detected in alveolar cells after PGIS gene transfer. In addition, intense immunoreactivity for PGIS was observed in the bronchial epithelium near the pulmonary vasculature. No major adverse effects of gene expression were observed in the transfected lung segments.

Lung Tissue Level of 6-keto-PGF₁α
Lung tissue content of 6-keto-PGF₁α in MCT rats was significantly increased 2 days (999±173 versus 534±103 pg/mg protein, P<0.05) and 7 days (1045±133 versus 716±68 pg/mg protein, P<0.05) after a single transfer of PGIS expression plasmid (Figure 5). Lung 6-keto-PGF₁α levels remained elevated 14 days after PGIS gene transfer, although this was statistically not significant.

Survival Analysis
Kaplan-Meier survival curves demonstrated that MCT rats transfected with PGIS gene had a significantly higher survival rate than those transfected with control vector alone (33% versus 0% in 10-week survival, log-rank test, P<0.01, Figure 6). No definite adverse effects were detected after repeated transfer of PGIS gene.

Discussion
In the present study, we demonstrated that (1) intratracheal transfer of the human PGIS gene by use of HVJ-liposomes induced overexpression of PGIS protein in bronchial epithelium and alveolar cells in MCT rats, resulting in augmenting prostacyclin synthesis in the lungs; and (2) transfer of the PGIS gene ameliorated MCT-induced pulmonary hypertension and pulmonary vascular remodeling. Finally, we demonstrated that (3) repeated transfer of the PGIS gene improved survival in MCT rats without definite adverse effects.

Patients with PPH have significantly decreased production of prostacyclin relative to that of thromboxane. Recently, PGIS expression has been shown to be particularly decreased in remodeled pulmonary arteries containing plexiform lesions in patients with PPH. These findings raise the possibility that impaired prostacyclin synthesis resulting from decreased PGIS may be implicated in the pathogenesis of PPH. In fact, continuous intravenous infusion of prostacyclin markedly lowers pulmonary vascular resistance in patients with PPH and improves survival beyond that attained with conventional therapy alone. However, prostacyclin is metabolized rapidly, requiring continuous intravenous administration. Quality of life still remains an important issue to be resolved. Therefore, it would be more desirable to enhance endogenous prostacyclin production.

In the present study, transfer of human PGIS gene to the lung could be achieved by intratracheal injection of HVJ-liposome complex. Intense immunoreactivity for PGIS was
observed predominantly in bronchial epithelium and alveolar cells. Earlier studies have shown that HVJ-liposomes have strong affinity for bronchial epithelium and alveolar cells.\textsuperscript{20,21} Recently, Saeki et al\textsuperscript{15} developed a novel anionic liposome with a lipid composition similar to the AVE, which showed up to a 10-fold improvement in transduction efficiency in vivo over conventional HVJ-liposomes. Thus, in the present study, this HVJ-AVE liposome was used for successful intratracheal gene transfer. The human PGIS gene discovered in our institute was used in the present study because the cDNA for human PGIS has high identity with its rat counterpart.\textsuperscript{3,22}

Recently, Champion et al\textsuperscript{23} showed that intratracheal gene transfer of calcitonin gene–related peptide to bronchial epithelial and alveolar cells attenuates chronic hypoxia-induced pulmonary hypertension in the mouse, suggesting that lung cell transduction with a vasodilator peptide may be sufficient to alter vascular function. Geraci et al\textsuperscript{12} showed that pulmonary prostacyclin synthase overexpression in transgenic mice protects against development of hypoxic pulmonary hypertension. Because they chose to use the epithelial cell–specific promoter instead of a vascular system–specific promoter, PGIS gene was expressed in bronchial epithelial cells and alveolar cells but not in pulmonary vessels. Nevertheless, PGIS-expressing mice did inhibit a pulmonary hypertensive response to acute hypoxia. In clinical settings, inhaled aerosolized prostacyclin acts transepithelially with pulmonary selectivity and improves pulmonary hypertension and oxygenation.\textsuperscript{24,25} These findings suggest that transepithelial delivery of prostacyclin to the vasculature may have beneficial effects on pulmonary hemodynamics. In the present study, lung tissue content of 6-keto-PGF\textsubscript{1α} was significantly increased for at least 1 week after transfer of PGIS gene, suggesting increased prostacyclin synthesis in the lung. The consequence of this synthesis in rats injected with MCT

Figure 4. Immunohistochemical demonstration of PGIS in lungs of MCT rats after PGIS gene transfer. Intense immunostaining for PGIS was observed in distal bronchial epithelium (A, arrows), in alveolar cells (B, arrows), and bronchial epithelium near pulmonary vasculature (C, arrows) after transfer of PGIS expression plasmid, which was hardly observed in those with control vector (D, E). Magnification ×400. Abbreviations as in text.

Figure 5. Lung tissue levels of 6-keto-PGF\textsubscript{1α} in MCT rats after single injection of PGIS expression plasmid (MCT-PGIS) or control vector (MCT-CON). Data are mean±SEM. *P<0.05 vs MCT-CON.

Figure 6. Kaplan-Meier survival curves showing that MCT rats transfected with PGIS expression plasmid (MCT-PGIS) have a significantly higher survival rate than those treated with control vector (MCT-CON) (log-rank test, P<0.01).
was a significant decrease in mean pulmonary arterial pressure and total pulmonary resistance. Importantly, the improvement in pulmonary hemodynamics was not accompanied by systemic hypotension, paralleling earlier results with gene transfer of endothelial nitric oxide synthase to the lung.26,27 Considering increased PGIS in the bronchial epithelium near the pulmonary vasculature, it is possible that endogenous prostacyclin produced by epithelial PGIS acts transepithelially with pulmonary vascular selectivity and ameliorates MCT-induced pulmonary hypertension.

Histological examination of MCT rats revealed that PGIS gene transfer inhibited an increase in the medial wall thickness of peripheral pulmonary arteries. In MCT-injected rats, endothelial cell injury caused by MCT activates platelets and vasoconstrictive factors, resulting in pulmonary hypertension and pulmonary vascular remodeling.28 Given the known potent vasoprotective effects of prostacyclin, such as vasodilation, antiplatelet aggregation, and inhibition of smooth muscle cell proliferation,1,2 it is interesting to speculate that endogenous prostacyclin produced by epithelial PGIS overexpression may act as a paracrine factor in the regulation of progressive pulmonary vascular remodeling in MCT rats.

Single gene transfer with HVJ-liposomes induced overexpression of PGIS and increased production of 6-keto-PGF<sub>1α</sub>, for at least 1 week. The HVJ-liposome method has been shown to be less toxic and less immunogenic than viral vectors such as adenovirus and herpesvirus because HVJ-liposomes are prepared after ultraviolet irradiation of the virus. Thus, MCT rats received repeated administration of the HVJ-liposome complex with PGIS gene every 2 weeks. Interestingly, repetitive transfer of PGIS gene significantly improved survival in MCT rats without definite inflammation in the lungs. Thus, PGIS gene therapy may be a promising treatment for severe pulmonary hypertension. However, the initial success of PGIS gene therapy reported here should be confirmed by long-term experiments, and extensive toxicity studies in animals are needed before clinical trials.

Conclusions

Intratracheal transfer of the human PGIS gene with HVJ-liposomes augmented pulmonary prostacyclin synthesis, inhibited MCT-induced pulmonary hypertension, and thereby improved survival in MCT rats. PGIS gene therapy may be a new therapeutic strategy for the treatment of pulmonary hypertension.

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References

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