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Transplantation of Autologous Endothelial Progenitor Cells May Be Beneficial in Patients With Idiopathic Pulmonary Arterial Hypertension

A Pilot Randomized Controlled Trial

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Objectives The goal of this study was to investigate the feasibility, safety, and initial clinical outcome of intravenous infusion of autologous endothelial progenitor cells (EPCs) in patients with idiopathic pulmonary arterial hypertension (IPAH).

Background Experimental data suggest that transplantation of EPCs attenuates monocrotaline-induced pulmonary hypertension in rats and dogs. In addition, clinical studies suggest that autologous progenitor cell transplantation is feasible and safe in patients with ischemic diseases.

Methods We conducted a prospective, randomized trial comparing the effects of EPC transplantation plus conventional therapy with those of conventional therapy alone in patients with IPAH. The primary end point was change in the 6-min walk distance using a standardized protocol. The secondary end points were changes in hemodynamic variables as assessed by right heart catheterization.

Results After 12 weeks of follow-up, the mean distance walked in 6 min increased by 48.2 m in the cell infusion group (from 263 ± 42 m to 312 ± 34 m), and an increase of 5.7 m occurred in the conventional therapy group (from 264 ± 42 m to 270 ± 44 m). The mean difference between the 2 groups was 42.5 m (95% confidence interval 28.7 to 56.3 m, $p < 0.001$). The patients in the cell infusion group also had significant improvement in mean pulmonary artery pressure, pulmonary vascular resistance, and cardiac output. There were no severe adverse events with cell infusion.

Conclusions This preliminary study showed that intravenous infusion of autologous EPCs seemed to be feasible and safe, and might have beneficial effects on exercise capacity and pulmonary hemodynamics in patients with IPAH. (Safety and Efficacy Study of Transplantation of EPCs to Treat Idiopathic Pulmonary Arterial Hypertension; http://www.clinicaltrials.gov/ct/show/NCT00257413?order=1; NCT00257413). (J Am Coll Cardiol 2007;49:1566–71) © 2007 by the American College of Cardiology Foundation

Idiopathic pulmonary arterial hypertension (IPAH), formerly known as primary pulmonary hypertension (PPH), is a rare disorder of unknown etiology characterized by raised pulmonary artery pressures with pathological changes in precapillary pulmonary artery. The median life expectancy from the time of diagnosis in patients with this disease, before the availability of disease-specific (targeted) therapy (1–3), was 2.8 years through the mid-1980s. Modern treatment has markedly improved physical function and has extended survival, and the 5-year mortality rate is 50% (4). However, we still do not understand what initiates the disease or what allows it to progress. There is no cure for the disease (5). Thus, a novel therapeutic strategy is desirable.

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Impairment of vascular and endothelial homeostasis is thought to play a major role in the initiation and development of IPAH (5,6). Many of the perturbations associated with the endothelial dysfunction promote vascular remod-

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eling in addition to increasing pulmonary vascular tone (4). Thus, pulmonary endothelial cells may be a therapeutic target for the treatment of IPAH.

Endothelial dysfunction ultimately represents an imbalance between the magnitude of injury and the capacity for repair (7). A variety of evidence suggests that endothelial progenitor cells (EPCs) constitute one aspect of the endothelial repair process (7). The EPCs are a cell population that has the capacity to circulate, proliferate, and differentiate into mature endothelial cells, but have neither acquired characteristic mature endothelial markers nor formed a lumen (8). These cells express some, but not all, cell surface markers characteristic of mature endothelium, certain surface markers of hematopoietic cells, and transcription factors that identify them as precursor cells. Laboratory evidence suggests that these precursors participate in postnatal neovascularization and re-endothelialization (8–10). Experimental data have suggested that transplantation of EPCs attenuated monocrotaline-induced pulmonary hypertension in the rat model (11,12). Recently, Takahashi et al. (13) reported that transplantation of autologous EPCs gave significant improvement in mean pulmonary artery pressure, cardiac output, and pulmonary vascular resistance in the dog dehydromonocrotaline model. In addition, clinical investigations suggest that autologous progenitor cell transplantation is feasible and safe in patients with ischemic diseases (14–16).

The feasibility, safety, and efficacy of autologous EPC transplantation in patients with IPAH are unknown. Therefore, we investigated the feasibility, safety, and initial clinical outcome of intravenous infusion of autologous EPCs in patients with IPAH.

Methods

Patients. Sporadic patients with IPAH between 18 and 60 years of age of either gender were invited to participate in the study. The definition of IPAH was pulmonary hypertension unexplained by any secondary cause, on the basis of the criteria of the National Institutes of Health registry (17). The diagnosis of IPAH was based on clinical assessment, right heart catheterization, echocardiography, spiral computed tomography of the pulmonary arteries, pulmonary angiography, ventilation/perfusion lung scan, and complete lung function testing. Patients were included in the study if they were in New York Heart Association functional class II to III, and had a mean pulmonary artery pressure >30 mm Hg on right heart catheterization. Additional inclusion criteria included the ability to walk ≥50 m during a standardized 6-min walk test. Pulmonary hypertension as a result of heart disease, pulmonary disease, sleep-associated disorders, chronic thromboembolic disease, autoimmune or collagen vascular disease, HIV infection, liver disease, New York Heart Association functional class IV, major bleeding requiring blood transfusion, diabetes, renal dysfunction, and evidence for malignant diseases were excluded.

Abbreviations and Acronyms

EPC = endothelial progenitor cell

IPAH = idiopathic pulmonary arterial hypertension

MNC = mononuclear cell

PPH = primary pulmonary hypertension

VEGF = vascular endothelial growth factor

Study protocol. The primary end point of the randomized study was the change from baseline in exercise capacity at the end of the study. Exercise capacity was determined by the 6-min walk test. The secondary end points included hemodynamic variables (mean pulmonary artery pressure, pulmonary vascular resistance, and cardiac output) as assessed by right heart catheterization. After the 6-min walk test and right heart catheterization at baseline, all patients were randomized to receive either conventional therapy (oral calcium blockers, diuretics, nitrates, digoxin, oxygen, prostaglandin E1, warfarin, and sildenafil) or cell infusions plus conventional therapy. At our center, calcium blockers were administered empirically. In China, neither intravenous prostacyclin nor aerosolized iloprost was licensed for the treatment of pulmonary hypertension until April 2006. Randomization was performed on the basis of random numbers, and a completely random design was used in this trial.

Clinical data, medication, and safety data were prospectively collected. Patients were followed up every 4 weeks for 12 weeks, and staff were continuously available via telephone for adverse event monitoring. After 12 weeks, the 6-min walk test and right heart catheterization were repeated.

The study protocol was approved by the ethics review board of the First Affiliated Hospital, College of Medicine, Zhejiang University, China. Written informed consent was obtained from all participants, and all of the procedures were done in accordance with the Declaration of Helsinki and relevant policies in China.

Exercise capacity and hemodynamics. The 6-min walk test was performed in all patients using a standardized protocol in accordance with the American Thoracic Society Statement 2002 (18). Patients walked along an enclosed-level corridor, and length to first turnaround point was 40 m. Technicians did not escort but encouraged patients using standard phrases such as "You are going well," "Keep up the good work," and were instructed not to use other encouragement. All patients were told to use their own pace, but to cover as much ground as possible in 6 min.

In all patients, a Swan-Ganz catheter (Arrow Deutschland Gmbh, Erding, Germany) and an arterial catheter (Angiocath, BD, Sandy, Utah) were inserted into the right internal jugular and right radial artery, respectively. Hemodynamic measurements were performed in recumbent position. Continuous monitoring included heart rate, systemic and pulmonary artery blood pressures, and transcutaneous oxygen saturation. Additional parameters were pressures in wedge position and right atrium. Cardiac output was obtained using triplicate measurements with the thermodilution method (Agilent, Boeblingen, Germany). Pulmonary vas-

cular resistance and systemic vascular resistance were calculated according to the standard formulas. Acute hemodynamic changes, which constitute evidence of vasoreactivity, were not examined at our center.

Both the walk test and right heart catheterization were administered by personnel who were unaware of the study protocol.

Preparation and transplantation of EPCs. In patients receiving cell infusion, 250 ml of venous blood was collected immediately after random assignment. The EPCs were isolated, purified, ex vivo cultured, and characterized according to previously described techniques (7,16,19–22). Briefly, mononuclear cells (MNCs) were isolated from peripheral blood of patients by Ficoll density gradient centrifugation and cultured on fibronectin-coated (Chemicon, Temecula, California) dishes in Medium 199 (Sigma, St. Louis, Missouri) supplemented with 20% human serum drawn from each individual patient and vascular endothelial growth factor (VEGF, 50 ng/ml, Chemicon). After 5 days in culture, cells were detached with 0.5 mmol/l ethylenediaminetetraacetic acid, washed 3 times with heparinized saline before final resuspension in 10 ml heparinized saline. Filtration was carried out to prevent cell clotting and microembolization. The resulting cell suspension contained a heterogeneous population of progenitor cells. More than 90% of the cells show EPCs characteristics demonstrated by dil-acetylated low-density lipoprotein (Molecular Probes, Eugene, Oregon) uptake and lectin (UEA-1, Sigma) binding, as we previously described (20–22). They were further documented by showing the expression of vascular epithelium-cadherin (81 ± 7.9%), kinase-insert domain-containing receptor (80 ± 8.2%), CD34 (28.7 ± 6.9%), and AC133 (17.1 ± 8.1%) by flow cytometry. The number of EPCs was evaluated by an independent investigator. Ten milliliters of EPCs suspension (injected cells, mean ± SD 1.1 ± 0.6 × 10^7, range 0.4 to 2.2 × 10^7) was directly intravenously infused into the cell infusion group of patients for about 5 min.

Statistical analysis. Our earlier observational study had shown a 50-m increase in exercise capacity with cell infusion. On the basis of this result, we calculated that we would require a sample size of 26 patients to show a 50-m improvement on the primary end point 6-min walk distance with 80% statistical power. To allow for missing observations and random differences in the recruited patient population, 3 additional patients were added per group. Therefore, we proceeded with an objective of enrolling 32 patients with an interim analysis after 28 patients completed the study. The intention-to-treat principle was applied in the analysis, and for missing observations, the last observation carried forward was done. Data are presented as mean ± SD and 95% confidence intervals. Continuous variables between the 2 groups were analyzed using independent-samples *t* test. Discrete variables between the 2 groups were compared using the Fisher exact probability test. A p value of <0.05 (2-sided) was considered to indicate statistical significance. All statistical analyses were performed using SPSS version 12.0 (SPSS Inc., Chicago, Illinois).

Results

Between December 15, 2003, and June 30, 2005, 33 patients with IPAH were informed in detail about the procedure of EPC transplantation. Informed consent was obtained from 31 patients, who were enrolled into the trial, whereas 2 patients refused to take part in the trial without giving any explanation.

All enrolled patients had a mean pulmonary artery pressure >30 mm Hg. Of the 31 patients, 16 were randomized to conventional therapy (conventional therapy group) and 15 to cell infusion plus conventional therapy (cell infusion group). The demographic and baseline characteristics of 2 groups are shown in Table 1. The conventional therapy and cell infusion groups were well matched with respect to demographic and baseline characteristics.

One patient in the conventional therapy group opted out of the study 3 weeks after randomization. This was not the result of any serious adverse effect of medication. No symptoms, including algor, fever, presyncope, shortness of breath, hypotension, and increased jugular venous distension, were occurred in the per-injection period. The number and nature of adverse events in drug administration (listed in Table 2) were similar between the 2 groups.

Table 1 Demographic and Baseline Characteristics of the Patients in 2 Groups

Characteristics	Conventional Therapy (n = 16)	Cell Infusion (n = 15)	p Value
Age, yrs	36 ± 9	35 ± 12	NS
Gender, n			
Male	4	4	NS
Female	12	11	NS
NYHA functional class, n			
2	5	5	NS
3	11	10	NS
Duration of symptoms, months	24 ± 11	25 ± 9	NS
Medication, n			
Anticoagulant agents	15	14	NS
Calcium antagonists	13	13	NS
Nitrates	9	8	NS
Digitalis	4	3	NS
Diuretics	1	1	NS
Prostaglandin E1	11	10	NS
Sildenafil	6	5	NS
Exercise capacity, m	264 ± 42	263 ± 42	NS
Mean pulmonary artery pressure, mm Hg	58 ± 9	57 ± 10	NS
Pulmonary vascular resistance, dyne·s·cm^{-5}	1,067 ± 220	1,091 ± 340	NS
Cardiac output, l/min	3.7 ± 0.6	3.7 ± 0.5	NS

Values are mean ± SD or number of patients.
NS = not significant; NYHA = New York Heart Association.

Table 2 Frequency of Adverse Effects During the Trial Period*

Effects	Conventional Therapy (n = 16)	Cell Infusion (n = 15)	p Value
Backache	3	1	0.600
Chest pain	2	4	0.378
Headache	3	1	0.600
Leg pain	1	3	0.333
Insomnia	4	2	0.394
Numbness of hands and feet	0	2	0.226
Anorexia	5	3	0.685
Nausea and vomiting	4	1	0.333
Abdominal discomfort	2	0	0.484
Constipation	3	1	0.600
Diarrhea	2	4	0.378
Giddiness	4	2	0.394

*Number of patients with adverse event.

The changes in the exercise capacity and hemodynamic variables from baseline to week 12 are listed in Table 3. After 12 weeks of follow-up, the mean distance walked in 6 min was increased by 48.2 m in the cell infusion group (from 263 ± 42 m to 312 ± 34 m), and an increase of 5.7 m occurred in the conventional therapy group (from 264 ± 42 m to 270 ± 44 m), with a mean difference of 42.5 m (95% confidence interval 28.7 to 56.3 m; $p < 0.001$).

Comparisons of the 2 groups showed that the patients in the cell infusion group also had significant improvement in mean pulmonary artery pressure, pulmonary vascular resistance, and cardiac output. The mean changes in mean pulmonary artery pressure for the cell infusion and conventional therapy groups were −4.5 and −0.4 mm Hg, respectively ($p = 0.001$), the mean changes in pulmonary vascular resistance were −185.4 and −27.8 dyne·s·cm^{-5}, respectively ($p = 0.002$), and the mean changes in cardiac output were 0.38 and 0.06 l/min, respectively ($p = 0.021$).

Discussion

To the best of our knowledge, this is the first pilot clinical study to assess the safety and efficacy of transplantation of autologous adult EPCs from peripheral blood in patients with IPAH. Here were present preliminary clinical data suggesting that transplantation of EPCs was feasible and safe in the patients. The intravenous infusion of EPCs was associated with increases in the 6-min walk distance and improvements in hemodynamics. The 6-min walk test is a reliable tool for the assessment of exercise capacity in patients with IPAH (2,23). It is suggested that the transplantation of autologous EPCs may have beneficial effects on exercise capacity and pulmonary hemodynamics in patients with IPAH.

Pulmonary vascular endothelial dysfunction was thought to play a major role in the initiation and development of IPAH (5,6). This endothelial dysfunction ultimately represents an imbalance between the magnitude of injury and the capacity for repair (7). There is strong evidence showing that EPCs may play an important role in endothelium maintenance, being implicated in both re-endothelialization and neovascularization (8–10,24–27). The possibility for infusion of autologous EPCs has been explored recently for the neovascularization and repair of ischemic organs. A recent study performed in patients with limb ischemia showed that intramuscular injection into the gastrocnemius of progenitor cells derived from bone marrow resulted in significant improvements in limb perfusion (14). Other clinical studies described the capacity of ex vivo expanded autologous EPCs from blood and/or bone marrow for the repair of human myocardium after infarction (15,16).

Therefore, transplanted EPCs may replenish endothelial cells, which could enhance the capacity for endothelial repair. Indeed, green fluorescent protein-expressing EPCs were incorporated into pulmonary arterioles and capillaries in rats and differentiated into mature endothelial cells 3 days after transplantation (11). Zhao et al. (12) reported that bone marrow-derived EPCs are involved in vascular regeneration in experimental pulmonary arterial hypertension. In experimental rats, the delivery of syngeneic endothelial-like progenitor cells from bone marrow nearly completely prevented the increase in pulmonary systolic pressures at 3 weeks after monocrotaline (12). Similarly, the delivery of these progenitor cells significantly reduced right ventricular hypertrophy (12). In rats receiving cells, there was a marked improvement in the appearance of the lung microvasculature, with preservation of arteriolar continuity and enhanced capillary perfusion (12). In addition, in experimental dogs with dehydromonocrotaline-induced pulmonary hypertension, transplantation of autologous EPCs from peripheral blood gave significant improvements in mean pulmonary artery pressure, cardiac output, and pulmonary vascular resistance (13). Histologic evaluation showed both improve-

Table 3 Changes in the Exercise Capacity and Hemodynamic Variables From Baseline to Week 12*

	Change From Baseline			
	Cell Infusion	Conventional Therapy	Mean Difference	95% Confidence Interval
6-min walk distance (m)	48.2 ± 17.1	5.7 ± 20.3	42.5	28.7 to 56.3
Mean pulmonary artery pressure (mm Hg)	−4.5 ± 3.4	−0.4 ± 2.4	−4.0	−6.2 to −1.9
Pulmonary vascular resistance (dyne·s·cm^{-5})	−185.4 ± 150.8	−27.8 ± 98.0	−157.6	−250 to −65
Cardiac output (l/min)	0.38 ± 0.43	0.06 ± 0.30	0.32	0.05 to 0.59

*Changes from baseline are presented as mean ± SD; 95% confidence intervals are for comparisons between the 2 groups. A confidence interval that does not contain zero indicates statistical significance.

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ments in the medial thickness of the small pulmonary artery and neovascularization of the lung tissue (13). On the other hand, Nagaya et al. (11) reported that the relatively small benefit could be obtained in response to EPCs therapy in monocrotaline-induced pulmonary hypertension in rats. It seems that the result might not reflect the true therapeutic potential of this cell therapy because these cells were isolated from human cord blood and used in a rat model. Because of important interspecies differences, these xenogenic cells may not be as well suited for pulmonary vascular repair as autologous progenitor cells (28). It is suggested that autologous EPCs may be more suitable for pulmonary vascular repair than xenogenic cells. In the present study, we seemed to transform the results from animal models to a safe clinical setting.

However, what remains unknown is how many cells are needed or how often cell transplantation may be more suitable for pulmonary vascular repair in patients with IPAH. A critical question for the EPCs therapy is the number of cells (7). One of the approaches for overcoming this problem is using umbilical cord blood (26). Another is mobilizing EPCs by cytokines, growth factors, or drugs (21,27,29). Furthermore, recent studies have shown a significant expansion of EPCs after ex vivo transfection with adenovirus-encoding VEGF, and VEGF gene transfer in vivo has been shown to mobilize EPCs in human subjects (30,31). Apparently, these include the need for viral or other vectors to deliver the genes, problems with short biological half-lives of the gene products, problems delivering the precise dose required, and problems with significant safety concerns (32). Overall, potent expansion of EPCs ex vivo will be required in the future.

Another major question is how to deliver the cells most efficiently. In the present trial, EPCs were directly intravenously infused into the patients with IPAH. Intravenously administered hematopoietic cells have been shown to be attracted to sites of cerebral injury (33). Kawamoto et al. (34) also reported that intravenously injected EPCs accumulated in ischemic myocardium after acute myocardial infarction. In addition, intravenously administered EPCs were incorporated into pulmonary arterioles and capillaries in rats and differentiated into mature endothelial cells (11). These findings suggest that progenitor cells have the ability to "sense" injured tissues. On the other hand, it was reported that the lung parenchyma of pulmonary hypertension model dogs was injected with autologous EPCs using a bronchoscope (13). Up to the present, it remains unknown whether infusion into the lung parenchyma has more beneficial effects than an intravenous administration on pulmonary arterial hypertension in human EPC transplantation.

Primum non nocere (first do no harm) is, as always, our overriding concern (32). Fortunately, there were not any severe complications related to cell infusion during this study. Autologous cell therapy begins with the premise that cellular repair occurs naturally in the human body and that enhancing the process should be safe. However, one theoretical safety concern regarding the intravenous delivery route was that cells administered this way may result in pulmonary arterial or arteriolar obstruction. Another major safety concern was the possibility of ex vivo contamination of the specimen. Infected or contaminated cells could potentially lead to devastating consequences (32). As such, all cell trial protocols should use good manufacturing practice facilities to minimize the risk.

Study limitations. The major limitation of this study was that it was not a double-blind, placebo-controlled trial. The placebo effect of an uncontrolled, unblended trial may be significant, particularly with regard to exercise capacity. The ethics review board refused to perform a double-blind, placebo-controlled trial. If there were a blinded control group in the clinical study, 250 ml of venous blood should be collected from all control patients. However, the valued blood in essence had to be thrown away afterward; therefore, we could not design this study as a double-blind, placebo-controlled trial because of ethical considerations. Fortunately, both the walk test and right heart catheterization were administered by personnel blinded to the study protocol.

Second, acute hemodynamic changes, which constitute evidence of vasoreactivity, were not always examined, and calcium channel blockers were usually administered empirically by respective physicians at our center. Most of the patients were on calcium antagonists, and the baseline data concerning pulmonary vascular reactivity were not provided in this work. In fact, calcium channel blockers have sustained hemodynamic improvement in a small subset (<25%) of the patients. In addition, there may be a potential imbalance in the groups between responders to calcium channel blockers, and so the observed effects in the present study may be confounded.

Third, the trial was conducted on a small number of patients with IPAH enrolled in this study, and the follow-up duration was relatively short. Some future studies with larger patient numbers will be required to establish long-term safety and efficacy of the cell infusion.

Finally, although EPCs effects in IPAH patients may be related to endothelium repair and neovascularization, the present clinical study cannot directly disclose the cellular mechanisms associated with the efficacy in patients with IPAH. In addition, we expanded circulating EPCs ex vivo, which predominantly showed expression of endothelial marker proteins but may also contain contaminating other progenitor cell populations.

Conclusions

Taken together, intravenous infusion of autologous EPCs seemed to be feasible and safe, and might beneficially affect exercise capacity and pulmonary hemodynamics in patients with IPAH, suggesting a new therapeutic option for patients with IPAH. Our data also provide enough proof for further investigation of EPCs and its role in IPAH patients.

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