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DISCUSSION

DR YVES D'UDEKEM D'ACOZ (Victoria, Australia): There is a saying that you should not do a bad Fontan, you should do only a good Fontan. And I think that I am still of the same opinion after having heard your excellent presentation. I think we needed the numbers you presented, it was very interesting. But when I look at your numbers, 25% of your patients died within 5 years and another 25% suffer from failure because you have 1 patient suffering from protein-losing enteropathy and 2 patients in NYHA class III. And I think we all know what it means to be a failing Fontan: these patients are very limited. So if you have 50% chance of success, what do you think that we should offer to the patient having seen these results? Should we offer heart transplantation or should we offer Fontan on one lung?

DR FUJII: I think I can understand your concern, but there is a very specific situation in Japan. Heart transplantation for children has been inhibited for a long time. Recently, heart transplantation for children became legal, but we still cannot find any heart transplantation donor for young children because of the social situation in Japan. I think it is spiritual or religious kind of thing. So we had no other choice. This is why these patients underwent one-lung Fontan operation.

Actually, the mortality is higher than the two-lung Fontan operation. The mortality is 25%. But some patients can spend an almost normal social life. The last patient I presented had survived for 19 years and can work as an office worker without any symptoms. So we have to think about the one-lung Fontan operation, I think.

DR SANO: I will answer your questions. Most of these patients are of the period where we are not allowed to do transplant, but it is clear that these one-lung patients who had the impaired ventricular function are very worse. So these patients may be a candidate of transplant. But the patient who had the reasonably good ventricular function, even these patients had one-lung Fontan, the long-term result is not bad. And I think that even transplant after the failed Fontan is not a very good long-term result.

Dr Photiadis: Right.

Dr Gaynor: So what shunt, what operation would you do for that patient?

Dr Photiadis: Right now I think there is no data. Our data do not show it, and there are no other data, so we would still use the B-T shunt.

Dr. S. Sano *(Okayama, Japan)*: Yes, a Sano would be probably a reasonable alternative.

Dr Gaynor: That is the one finding that did come out in your studies.

Dr E. Bove *(Ann Arbor, MI, USA)*: I enjoyed your paper, and I agree with your conclusion, but I rise to support what Dr. Gaynor said, and I think that we have to be very careful how we interpret data. It is a retrospective study, and if I understood from your abstract, it was surgeon preference which was not very clear. Does that mean one surgeon did one technique, another surgeon did a different one so that each surgeon decided preoperatively what he felt would be the best shunt? Additionally, looking at postoperative risk factors and showing that the Aristotle scores are equal does not have the same validity as a prospective randomized trial to reach the conclusion as you have reached. So even though I would like to agree with your conclusion, I think we have to be very careful how we interpret these data.

Dr Photiadis: You are absolutely right, but again, randomisation may not give you the whole answer. If we have a patient, say aortic atresia, mitral atresia, who is stable preoperatively and whom you randomly assign to the Sano shunt, and then you have another patient with aortic atresia and mitral atresia, same anatomic type, was not prenatally diagnosed, decompensation without closure of the duct and cannot be stabilised, if you look on just the anatomic type, they are the same, but with respect to preoperative risk in fact they are not. By means of the Aristotle score these differences can be detected, with one patient being assigned to the low-risk and the other one to the high-risk group.

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EDITORIAL COMMENT

Re: Does the shunt type determine mid-term outcome after Norwood operation?

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Keywords: Hypoplastic left heart syndrome • Norwood operation (modified Blalock–Taussig shunt) • Sano operation (right ventricle-pulmonary artery shunt)

There is always a debate as to which shunt is better in a Norwood procedure [1–4]. Some say the modified Blalock–Taussig (MBT) shunt is better than the right ventricle-pulmonary artery (RV-PA) shunt, while others say the RV-PA shunt is better than the MBT shunt. Photiadis *et al.* [1] compared their experience of MBT shunts with that of RV-PA shunts in 109 patients. They concluded that there was no significant difference in the survival rates between the two shunt types. However, the incidence of shunt-related interventions was significantly increased with the RV-PA (Sano) shunt group. Ohye *et al.* [2] reported the results of the first multicentre, randomized trial of the Norwood procedure with comparison of an RV-PA shunt and MBT shunt. They summarized that the 12-month transplantation-free survival was higher with the use of an RV-PA shunt than with the use of an MBT shunt. However, the RV-PA shunt was associated with a higher rate of unintended cardiovascular interventions and complications during the first 12 months after randomization. Their conclusion was that there was no significant difference between the two groups with respect to transplantation-free survival beyond 12 months.

The Norwood procedure using an MBT shunt creates pulmonary atresia from aortic atresia, while the idea of an RV-PA shunt is to create tetralogy of Fallot from aortic atresia. Postoperative management after the implantation of an MBT shunt in patients with pulmonary atresia was not easy because it is important to keep a balance between systemic and pulmonary circuits. Babies with an MBT shunt sometimes collapse suddenly in the ward or at home. However, almost no babies experience a sudden collapse in tetralogy of Fallot. While many surgeons and many centres report almost no or minimal mortality in patients with MBT shunt, and that it is easy to keep a balance between systemic and pulmonary circuits, the discharge mortalities in neonates after MBT shunts were 7.2 and 10.6%; in the Society of Thoracic Surgeons database [5] and in the European Society for Cardio-Thoracic Surgery database (http://www.eactscongenitaldb.org/index.php?LANG=en&level=1&struct=14), respectively. These data clearly show that the MBT shunt has a high mortality, and this is the reality of the situation.

Creating a tetralogy of Fallot instead of pulmonary atresia was the simple idea behind the RV-PA shunt when I started using the RV-PA shunt to treat the hypoplastic left heart syndrome. This was because postoperative management after the implantation of a MBT shunt was not easy.

We all worry about the adverse effects on RV function after a ventriculotomy; however, there are many papers that have demonstrated no adverse effects in the use of a RV-PA shunt [3, 6]. The site and size of the ventriculotomy as well as the size of the shunt are important. Initially, we created an RV hole by

using a knife and a pair of scissors and this was changed to a coronary puncher to create uniform and minimally sized holes. Furthermore, pulmonary blood flow is controlled using a clip on the graft with oxygen saturation staying at around 80–85%; on room air. These changes may affect long-term RV function. The authors and others also alluded to a higher rate of unintended cardiovascular interventions and complications with the use of the RV-PA shunt. A ring-enforced polytetrafluoroethylene (PTFE) graft is the option to avoid proximal graft stenosis [7]. Recently, we have begun to use ring-enforced PTFE grafts frequently instead of a ringless graft. By adopting these modifications, we may be able to decrease the mortality and morbidity after a RV-PA shunt.

All available data show no difference in mortality between the two different shunts. Some surgeons prefer to do an MBT shunt and some prefer to do an RV-PA shunt. Whichever shunt is used, more efforts should be made to improve mortality and morbidity.

Conflict of interest: none declared.

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Four-year clinical outcomes of the OLIVUS-Ex (impact of Olmesartan on progression of coronary atherosclerosis: Evaluation by intravascular ultrasound) extension trial

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ABSTRACT

Background: The previous OLIVUS trial reported a positive role in achieving a lower rate of coronary atheroma progression through the administration of Olmesartan, an angiotension-II receptor blocking agent (ARB), for stable angina pectoris (SAP) patients requiring percutaneous coronary intervention (PCI). However, the benefits between ARB administration on long-term clinical outcomes and serial atheroma changes by IVUS remain unclear. Thus, we examined the 4-year clinical outcomes from OLIVUS according to treatment strategy with Olmesartan.
Methods: Serial volumetric IVUS examinations (baseline and 14 months) were performed in 247 patients with hypertension and SAP. When these patients underwent PCI for culprit lesions, IVUS was performed in their non-culprit vessels. Patients were randomly assigned to receive 20–40 mg of Olmesartan or control, and treated with a combination of β-blockers, calcium channel blockers, glycemic control agents and/or statins per physician's guidance. Four-year clinical outcomes and annual progression rate of atherosclerosis, assessed by serial IVUS, were compared with major adverse cardio- and cerebrovascular events (MACCE).
Results: Cumulative event-free survival was significantly higher in the Olmesartan group than in the control group ($p = 0.04$; log-rank test). By adjusting for validated prognosticators, Olmesartan administration was identified as a good predictor of MACCE ($p = 0.041$). On the other hand, patients with adverse events ($n = 31$) had larger annual atheroma progression than the rest of the population (23.8% vs. 2.1%, $p < 0.001$).
Conclusions: Olmesartan therapy appears to confer improved long-term clinical outcomes. Atheroma volume changes, assessed by IVUS, seem to be a reliable surrogate for future major adverse cardio- and cerebrovascular events in this study cohort.

1. Background

Despite the widespread application of established medical therapies, extensive cardiovascular disease remains the most important cause of morbidity and mortality in patients with ischemic coronary disease [1–11]. Although angina pectoris is characterized by a clustering of cardiovascular disease risk factors, such as dyslipidemia, diabetes, and hypertension, optimal atheroma management is a key strategy for preventing subsequent cardiovascular events [1,4–10,12–16]. Prior intravascular ultrasound (IVUS) trials reported a slowing of coronary atheroma progression or regression with some medicines, however, the direct benefits between drug administration on long-term clinical outcomes and atheroma volume changes, assessed by IVUS, have not been well clarified [1,4–10,12–16].

The OLIVUS trial, using serial volumetric IVUS, reported a positive role in achieving a lower rate of coronary atheroma progression through the administration of Olmesartan, an angiotension-II

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receptor blocking agent (ARB), for stable angina pectoris (SAP) patients requiring percutaneous coronary intervention (PCI) [17]. According to treatment strategy with Olmesartan, we investigated the 4-year OLIVUS follow up data to evaluate the relation between atheroma volume change and clinical outcomes.

2. Methods

2.1. Patients and study design

The OLIVUS trial is a prospective, randomized, multicenter trial which examined the impact of Olmesartan on the progression of coronary atherosclerosis; evaluation by intravascular ultrasound (OLIVUS) [17]. Patients with hypertension and clinically stable angina pectoris scheduled for percutaneous coronary intervention (PCI) were enrolled. After PCI for their culprit lesions, IVUS was performed over 40 mm in their non-culprit vessels, defined as without angiographically documented coronary stenosis <50%, to determine plaque volume at baseline. Hemodynamically unstable patients, recent myocardial infarction within 4 weeks, ejection fraction <25%, and patients already on ACE inhibitors or ARBs were excluded from the trial. Patients were randomized to control or Olmesartan 10–40 mg titrated to maximally tolerated dose by 8 weeks. In addition, patients were treated with a combination of β-blockers, calcium channel blockers, diuretics, nitrates, glycemic control agents and/or statins per physician's guidance. After 12–16 months, IVUS of the originally examined coronary artery was performed during the routine follow-up angiogram. The extended-OLIVUS trial increased the follow-up period of the OLIVUS trial to evaluate associations between clinical prognosis, coronary atheroma changes and Olmesartan treatment. The study protocol was approved by all participating institutional review boards and all patients provided written informed consent. The primary endpoint was the incidence of major adverse cardio- and cerebrovascular events (MACCE), including the composite of death from cardiac or cerebral causes, myocardial infarction, stroke, rehospitalization due to unstable or progressive angina according to the Braunwald unstable angina classification and the Canadian Cardiovascular Society angina classification, deterioration of heart function or renal failure. Stroke was diagnosed based on the presence of a neurologic deficit confirmed by computed tomography or magnetic resonance imaging. Outcome data were collected by serial contact with the patients or their families until July 31, 2011. Medical records of patients who died or who were treated at participating hospitals were analyzed.

2.2. Intravascular ultrasound

IVUS studies were performed using a commercially available imaging system with a 40-MHz mechanical transducer ultrasound catheter (Boston Scientific Corporation, Natick, MA). Using automated pullback (0.5 mm/s), ultrasound images were obtained and recorded for off-line quantitative analysis. The images were digitized and three-dimensional volumetric analysis was performed using Simpson's method (EchoPlaque, Indec Systems, Mountain View, CA). Measurements included vessel, lumen and atheroma volumes (ATV) over the 40 mm segment in the non-PCI-culprit vessels. To standardize for vessel size, percent atheroma volume (%ATV), defined as plaque volume divided by vessel volume, was also calculated. The serial progression rate of atherosclerosis was compared with change in absolute atheroma volume and change in percent atheroma volume, measured by (follow-up ATV − baseline ATV)/baseline ATV, and (follow-up %ATV − baseline %ATV)/baseline %ATV, respectively. All analytic methods were previously reported [17,18].

Table 1
Baseline patient characteristics and medications.

	Control ($n=121$)	Olmesartan ($n=126$)	p
Gender (male, %)	68	76	ns
Age (years)	68.4 ± 8.8	67.8 ± 8.7	ns
Smoking (%)	31	34	ns
Diabetes (%)	35	31	ns
Previous MI (%)	13	15	ns
Aspirin (%)	100	100	ns
β-Blocker (%)	13.2	12.7	ns
Calcium channel blockers (%)	49.6	41.3	ns
Statins (%)	74.0	71.4	ns
Oral diabetic agents (%)	17.3	19.8	ns
Insulin (%)	7.1	5.6	ns

2.3. Statistical methods

Analyses were performed using SPSS 11 software (SPSS Inc., Chicago, IL). Laboratory and ultrasound parameters were reported as the mean value ± SD. Continuous variables are expressed as means ± SD. Data from two independent groups were compared using a t-test or Wilcoxon rank-sum test. Intra-group data were analyzed using a paired t-test or the Wilcoxon signed-rank test. Categorical data were tabulated as frequencies and percentages and compared using the χ^2 test or Fisher's exact test. Event-free survival probabilities for MACCE were estimated using the Kaplan–Meier method and group differences were assessed using a log-rank test. Unadjusted hazard ratios for variables, namely administration of Olmesartan, statin, age, gender, atheroma volume changes, baseline percent atheroma volume, hypertension, diabetes, smoking, prior history of coronary artery disease and baseline LDL-C values, were calculated using the Cox proportional hazards model. A two-sided p-value of <0.05 was considered significant.

3. Results

Between February 2006 and August 2007, 247 patients with stable angina pectoris patients undergoing PCI were enrolled in this trial. Prognostic data were fully documented during the entire follow-up period (mean duration, 4.1 ± 1.3 years). During follow up, 15 patients in the control group and 17 patients in the Olmesartan group dropped out of the trial because of MACCE, laboratory abnormality or having withdrawn consent. Even though serial volumetric IVUS analyses were completed in 205 patients, vital status was ascertained in 233 (93.3%) patients at the end of the study. Of the 118 (93.6%) patients taking Olmesartan at the end of the study, 107 (84.9%) were on the full dose (20–40 mg), with only 4 (3.2%) on a reduced dose.

3.1. Patient characteristics and blood pressure changes

Patient characteristics and medications are summarized in Tables 1 and 2. All data are identical between the control and Olmesartan groups. Serial changes in blood pressure are presented in Fig. 1. In this trial, blood pressure control was at the physician's discretion except for administration of Olmesartan. While significant improvement in blood pressure, LDL/HDL cholesterol and glycemic control were observed in both groups, there was no significant difference between the control and Olmesartan group.

3.2. Volumetric IVUS analysis

Significant development of atheroma volume (ATV) and percent atheroma volume (%ATV) was found in the control group between baseline and 14-months follow-up (from 208.8 ± 151.5 to 215.9 ± 156.8 (mm^3), $p<0.01$ for ATV, from 40.6 ± 10.8 to

Table 2
Blood parameters of patients at baseline and 4-years follow-up.

	Baseline			Follow-up		
	Control	Olmesartan	*p*-Value	Control	Olmesartan	*p*-Value
Total cholesterol (mg/dl)	185.9 ± 34.3	183.3 ± 29.6	0.554	183.8 ± 37.0	181.4 ± 30.4	0.941
HDL-C (mg/dl)	50.4 ± 12.6	47.1 ± 12.7	0.073	56.1 ± 15.2	52.6 ± 13.3	0.084
Triglyceride (mg/dl)	142.4 ± 64.6	163.9 ± 126.4	0.131	140.4 ± 73.9	158.7 ± 81.0	0.093
LDL-C (mg/dl)	108.0 ± 30.2	106.8 ± 24.8	0.405	101.7 ± 30.8	101.2 ± 26.2	0.915
Body Mass Index (kg/m^2)	23.9 ± 3.5	24.7 ± 3.2	0.091	23.7 ± 3.0	24.3 ± 4.3	0.270
Creatinine (mg/dl)	1.00 ± 0.41	0.99 ± 0.25	0.844	0.92 ± 0.44	0.97 ± 0.29	0.153
e-GFR (ml/min/1.73 m^2)	57.9 ± 19.2	59.6 ± 17.5	0.517			
HbA1c (%)	5.9 ± 1.2	6.1 ± 1.1	0.358	5.7 ± 0.9	5.9 ± 0.9	0.242
BNP (pg/dl)	49.8 ± 47.2	45.1 ± 29.7	0.482	46.9 ± 67.9	37.4 ± 32.3	0.211

* $p < 0.01$.
† $p < 0.05$ between baseline to follow-up.

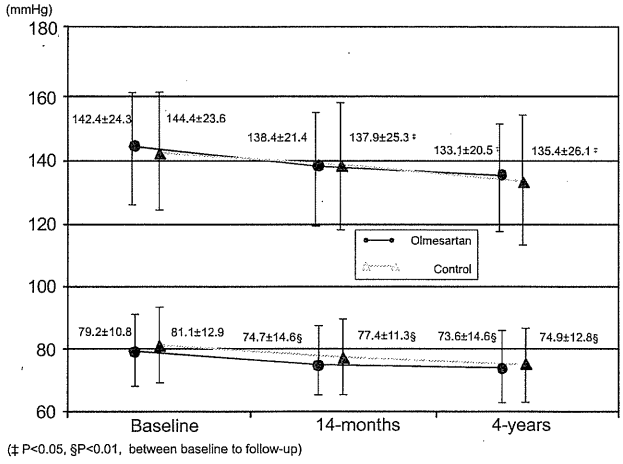

Fig. 1. Serial changes of blood pressure in the study period.

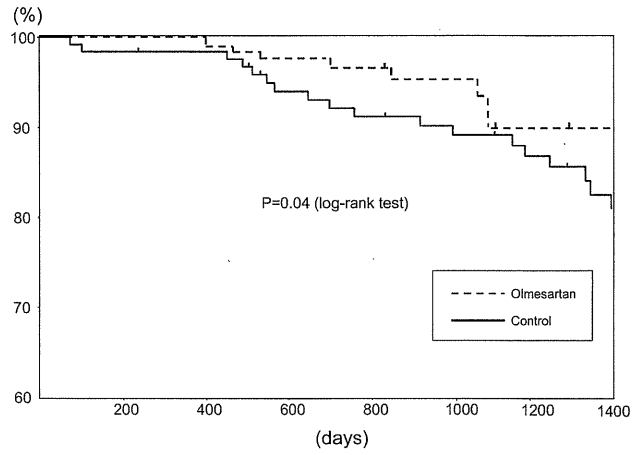

Fig. 2. Cumulative event-free from cardio or cerebrovascular death, myocardial infarction, stroke, angina, or heart/renal failure.

41.7 ± 11.5 (%), $p < 0.05$ for %ATV). However, there was no difference between ATV and %ATV in the Olmesartan group (230.2 ± 151.7 to 227.6 ± 145.8 (mm^3) for ATV, 43.8 ± 10.2 to 43.7 ± 10.4 (%) for %ATV, p = ns for all). Furthermore, serial change in ATV and %ATV were significantly lower in the Olmesartan group than in the control group (0.6 ± 12.9 vs. 5.4 ± 15.5 (%) $p = 0.016$ for ATV, −0.7 ± 13.6 vs. 3.1 ± 12.5%, $p = 0.038$ for %ATV, respectively). However, in this trial, there was no statistically significant correlation between blood pressure reduction and plaque progression rate.

3.3. *Major adverse cardio- and cerebrovascular events (MACCE)*

Adjudicated major adverse cardio- and cerebrovascular events are summarized in Table 3. While there was no difference in terms of individual cardio- and cerebrovascular event between the two groups, the composite event rate of cardio- and cerebrovascular death, MI, stroke, angina, or heart/renal failure was significantly lower in the Olmesartan group ($p = 0.041$). Cumulative event-free from MACCE was significantly higher in the Olmesartan group than in the control group ($p = 0.04$, log-rank test; Fig. 2, Hazard ratio 0.41 (95% CI: 0.18–0.91, Relative risk reduction = 0.54)). Estimates of hazard ratios for MACCE are presented in Fig. 3. Advanced age, prior history of coronary artery disease, 3-vessel disease, poorly controlled diabetes, higher %ATV increase and higher original %ATV were identified as poor predictors of MACCE. However, administration of Olmesartan and statins were selected as predictors for reduced MACCE. Comparison of serial atheroma progression rate for patients with adverse events ($n = 31$) and the rest of the population is presented in Fig. 4. Patients with adverse events had larger annual atheroma progression than the rest of the population ($p < 0.001$). During the follow-up period, there were no adverse events attributed to Olmesartan.

Table 3
Four-years adjudicated major cardiovascular events.

	Control (n = 121)	Olmesartan (n = 126)	p
Composite of cardio or cerebrovascular death, MI, stroke, angina, or heart/renal failure	17.4	8.0	0.04
Death (all cause)	3.3	3.2	0.95
Death (cardio or cerebrovascular)	1.7	0.8	0.51
Nonfatal myocardial infarction	0.8	1.6	0.59
Nonfatal stroke	1.7	0	0.15
Unstable angina/increasing angina	10.7	4.8	0.10
(Culprit related/new de novo coronary lesions)	(5.0/5.7)	(3..2/1.4)	
Deterioration of heart/renal failure	2.5	0.8	0.30

MI indicates myocardial infarction.

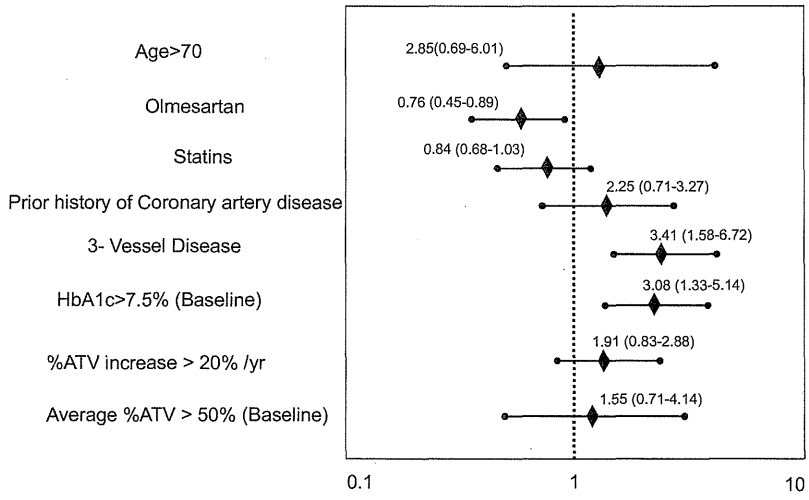

Fig. 3. Hazard ratio for major adverse cardio- and cerebrovascular events (MACCE) are presented. Hazard ratio and 95% CI value are listed.

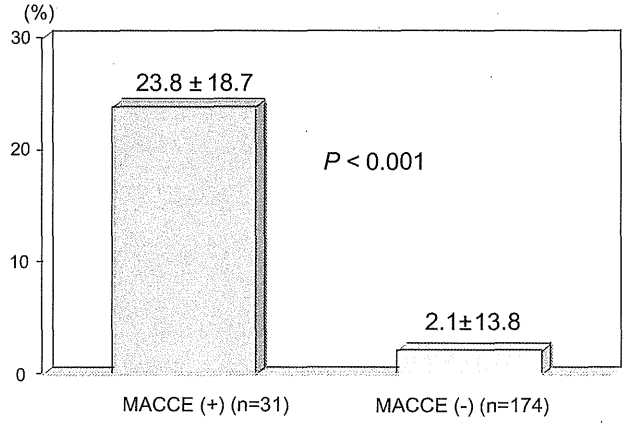

Fig. 4. Comparison of major adverse cardio- and cerebrovascular events (MACCE) and annual atheroma progression.

4. Discussion

The present study demonstrated that administration of Olmesartan was associated with reduced incidence of long-term cardio- and cerebrovascular events in patients with hypertension and stable angina pectoris after PCI. In addition, the 14-months IVUS follow-up showed that patients who had greater atheroma progression had an increase in subsequent cardio- and cerebrovascular events. In previous IVUS trials, interventions that targeted established risk factors demonstrated favorable effects on the rate of progression of coronary atherosclerosis. In the initial stage of the current trial, significant plaque regression was observed in patients receiving Olmasartan, an ARB, compared with the control group during the 14-months follow-up period. The extended-OLIVUS trial demonstrated sustained reduction in incidence of composite cardiovascular complications during the 4-years while receiving Olmesartan. Currently, ARBs are widely used for the treatment of hypertension. They also have beneficial effects on hypertension-related cardiovascular end organ damage, possibly due to reduction of oxidative stress and inflammation [19–21]. While there are several ARBs available in the clinical setting, Olmesartan is thought to have a significantly stronger antihypertensive effect than other ARBs with their respective starting doses [19–23]. In addition, previous studies as well as the OLIVUS trial have reported the potential decrease of atheromatous plaque burden in human coronary arteries after administration of Olmesartan, compared with the control group [17]. Furthermore, previous trials reported a significant reduction in the incidence of stroke and angina pectoris in patients receiving ARBs [20]. Our study data show the corroborating efficacy for these medicines in terms of preventing the progression of atherosclerosis, even though the number of enrolled patients was relatively small. However, the underlying mechanisms as well as the clinical impact of ARBs remain a matter of ongoing debate. There was no significant difference in terms of changes of blood pressure. In this trial, control of blood pressure was left to physician's discretion except for administration of ARBs and ACE inhibitors, therefore, an incremental dose of other antihypertensive agents, such as β-blockers, calcium channel blockers and/or diuretics, may have contributed to the similarities in blood pressure control between the 2 groups. In the present trial, there was no statistically significant correlation between the degree of blood pressure reduction and plaque progression rate or event rate. This may suggest the potential manifold action of Olmesartan apart from the antihypertensive effect that might be beneficial, such as activity leading to plaque stabilization and reduction. In the present trial, however, there was no significant effect on the hard events including cardiac and cerebral deaths and myocardial infarction during the 4-years follow-up period. Most events were re-hospitalizations for unstable or progressive angina, therefore, death from cardiac causes, cardiac arrest, and myocardial infarction were less common. The effects of increasing disease burden on death or myocardial infarction were not evident in this analysis, which likely reflects the finding that there were few mortal events under the current optimal medical therapy [16,24]. These results are consistent with previous trials suggesting that effects on hard endpoints using ARB showed close to the significance compared with the control group [20,23].

In the current trial, Olmesartan and statins were selected as predictors of MACCE reduction. However, a high proportion of patients in our study were showing relatively lower LDL cholesterol level at baseline, and already being treated with lipid-lowering agents (57.0% in the control and 52.3% in the Olmesartan group), which might have minimized the differences in MACCE events seen between the two randomized groups than in previous trials [1–3,5,6,9,10,12,25,26]. In the present study, it may be important to note that atheroma volume, reflecting the interaction between the artery wall and atheroma throughout the imaged segment, did associate with the likelihood of having a clinical event.

Volumetric IVUS analyses were completed exclusively in entire vessels (more than 40 mm); therefore, these IVUS parameters may represent atheroma progression of coronary as well as cerebral arteries, since atherosclerosis progresses systemically. The results suggest that atheroma volume changes, assessed by serial IVUS, seem to be a reliable surrogate for future major adverse cardio- and cerebrovascular events in this study cohort [4,12,16,17,24,27–29].

The present analysis demonstrates a relationship between the progression of coronary atherosclerosis, as determined by IVUS, and the prospective risk for cardio- and cerebrovascular events. To diminish subsequent cardiovascular risk, therapeutic strategies designed to prevent or delay the progression of coronary disease is of great clinical importance. We believe this is the first clinical trial that shows the direct potential benefits between long-term drug administration on long-term clinical outcomes and atheroma volume changes, assessed by IVUS, using an ARB. Our study data may add another striking benefit to the ever-growing list of positive outcomes associated with Olmesartan administration.

5. Limitations

There are several limitations in our study. First, a small number of patients with stable angina pectoris were enrolled; therefore, some selection bias may exist. Second, the IVUS results showed relatively larger standard deviations, however, these are not unusual for this kind of study. In addition, a high proportion of patients in our study were already being treated with optimal lipid-lowering therapy, therefore, it may be difficult to show an effect in addition to that treatment.

6. Conclusions

These observations suggest that administration of Olmesartan was associated with reduced incidence of long-term cardio- and cerebrovascular events in patients with hypertension and stable angina pectoris after PCI. Atheroma volume changes, assessed by serial IVUS, seem to be a reliable surrogate for future major adverse cardio- and cerebrovascular events in this study cohort.

Conflict of interest

None.

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Original Article

CD44 expression in plexiform lesions of idiopathic pulmonary arterial hypertension

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Plexiform lesions in pulmonary arteries are a characteristic histological feature for idiopathic pulmonary arterial hypertension (IPAH). The pathogenesis of the plexiform lesion is not fully understood, although it may be related to endothelial cell dysfunction and local inflammation. CD44 is a cell adhesion molecule and it is also involved in angiogenesis, endothelial cell proliferation and migration. The expression of CD44 was examined in lung plexiform lesions obtained from patients with IPAH (IPAH group, $n = 7$) and pulmonary arterial hypertension associated with atrial septal defect (ASD-PAH group, $n = 4$). Expression of CD44 was detected in 49 out of 52 plexiform lesions (93%) from all patients in the IPAH group, whereas 31 plexiform lesions obtained from the ASD-PAH group lacked CD44 positivity by immunohistochemistry. In the IPAH group, CD44 was localized in the endothelial cells of microvessels within plexiform lesions and activated T cells in and around the lesions. Furthermore, T cell infiltration and endothelial cell proliferation activity were prominent in the plexiform lesions of the IPAH group, compared to those of the ASD-PAH group. These findings suggest that CD44 and activated T cell infiltration play an important role in the development of plexiform lesions particularly in IPAH.

Key words: CD44, endothelial cell, plexiform lesion, pulmonary arterial hypertension

Idiopathic pulmonary arterial hypertension (IPAH) is a rare disorder, characterized by sustained elevation of pulmonary arterial pressure without a demonstrable cause.[1] Although the discovery of the mutations in the gene encoding the bone morphogenetic protein receptor type II (BMPR2) in patients with familial pulmonary arterial hypertension (PAH)[2,3] and sporadic IPAH[4] emphasized the importance of BMP/TGF-β signaling, the pathogenesis of IPAH has not been fully clarified. The underlying mechanism is pulmonary vascular remodeling involving the pre- and intra-acinar pulmonary arteries: (i) constrictive lesions such as intimal thickening and medial hypertrophy and (ii) complex lesions characterized by plexiform lesions.[5] The plexiform lesion has been highlighted in pulmonary arteriopathy of IPAH, because it is not only a marker of severity or rapid progression of pulmonary hypertension,[6] but also has contributed to pathogenesis of the disease, particularly with regard to endothelial cell abnormalities and inflammation.

Morphologically, the plexiform lesion is a focal proliferation of endothelial channels lined by myofibroblasts, smooth muscle cells, and connective tissue matrix in an aneurysmal dilatation of muscular pulmonary artery branches associated with partial destruction of the vessel wall.[5,7] Previously, evidence of endothelial dysfunction,[8,9] one of the key pathogeneses of IPAH, as well as endothelial cell proliferation[7,10,11] and disordered angiogenesis[12] have been shown in plexiform lesions. In addition to endothelial abnormalities, frequent inflammatory infiltrates such as lymphocytes and macrophages have been identified in plexiform lesions,[7] suggesting that inflammatory mechanisms also play an important role in the pathogenesis of IPAH. This view has been supported by the frequent presence of autoantibodies such as circulating antinuclear antibodies and elevated circulating levels of proinflammatory cytokines[13] and chemokines[14] in IPAH patients. Moreover, increased fractalkine expression in PAH lungs and its receptor on circulating T cells[15] as well as increased expression of chemokine RANTES (regulated

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upon activation, normal T-cell expressed and secreted) predominantly in plexiform lesions associated with CD45-positive cell infiltrates[16] emphasize a role of inflammatory cell recruitment in this disorder.

CD44 is a member of the hyaluronate (HA) receptor family of cell adhesion molecules involved in leukocyte trafficking.[17,18] This molecule can mediate the adhesion of lymphocytes to vascular endothelial cells via binding of HA and the extravasation of activated T cells into sites of inflammation in mice[19] and in human autoimmune disease.[20] In addition, CD44 is involved in endothelial cell proliferation, migration and angiogenesis[21,22] that are the characteristic features of plexiform lesions in IPAH.

In light of these functions, we hypothesized that CD44 might be involved in the progressive vascular remodeling, particularly in plexiform lesions of patients with IPAH. The purpose of the present study was to begin an exploration of the potential roles of CD44 in the pathogenesis of IPAH by determining the immunohistochemical expression and localization of CD44 in the plexiform lesions and associated T cell recruitment.

MATERIALS AND METHODS

Case materials

Formalin-fixed, paraffin-embedded blocks of lung tissue displaying plexogenic pulmonary arteriopathy were retrieved from the archival collection of the Department of Pathology, National Cerebral and Cardiovascular Center, Suita, Japan, apart from two cases provided by the Department of Cardiovascular Medicine, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Science, Okayama, Japan. Seven cases of IPAH (6 women, 1 man, mean age 31 ± 5 years old, mean pulmonary arterial pressure (PAP) 64 ± 4 mmHg) were entered into the study.

To compare CD44 expression between idiopathic and non-idiopathic cases, four cases of PAH associated with atrial septal defect (ASD-PAH) (3 women, 1 man, mean age 28 ± 9 years old, mean PAP 63 ± 4 mmHg) were also included. Clinical classification of PAH patients was according to Dana Point Classification[23] and the patients' clinical information is presented in Table 1. The patients were all nonsmokers, and were without known risk factors or associated conditions of PAH[23] such as appetite suppressants, connective tissue diseases, human immunodeficiency virus infection, portal hypertension, congenital heart disease (except ASD-PAH cases), schistosomiasis, chronic hemolytic anemia or chronic parenchymal lung disease. All cases originated from autopsy material, except two lung specimens from Okayama University (one with IPAH and the other with ASD-PAH) obtained at the time of lung transplantation. Two blocks for each lobe of bilateral lungs were prepared randomly in each case. Plexiform lesions were identified and counted on hematoxylin and eosin stained sections. Normal lung tissues from three autopsy cases (1 woman, 2 men, mean age 52 ± 20 years old) with no cardiac or pulmonary disease were used as controls.

Immunohistochemistry

Each paraffin-embedded block including plexiform lesions was serially cut into three-micrometer thick sections. The sections were pre-incubated with blocking reagent to quench endogenous peroxidase activity, followed by incubation with the primary antibodies at room temperature, except when otherwise noted. The antibody for CD44 (1:25, Dako, Glostrup, Denmark) was incubated at 4°C overnight. The antibodies for CD31 (1:50, Dako) for endothelial cells, CD68 (1:50,

Table 1 Clinical classification and information of the PAH patients

Patients	Sex	Age	mPAP (mmHg)	CI (L/min/m^2)	Risk factors* for PAH	ANA	Iv PGI_2	Cause of death	Lung specimen
IPAH1	F	18	58	1.7	None	Negative	–	Right heart failure	Autopsy
IPAH2	F	32	62	1.6	None	Negative	+	Right heart failure	Autopsy
IPAH3	F	43	72	2.6	None	Negative	+	Alive (LTx)	LTx
IPAH4	F	26	78	1.6	None	Negative	–	Right heart failure	Autopsy
IPAH5	M	54	71	1.9	None	Negative	–	Right heart failure	Autopsy
IPAH6	F	12	45	3.0	None	Negative	–	Right heart failure	Autopsy
IPAH7	F	34	63	0.8	None	Negative	+	Right heart failure	Autopsy
ASD-PAH1	F	51	54	NA	CHD	NA	–	Sudden arrest	Autopsy
ASD-PAH2	M	13	71	NA	CHD	NA	–	Right heart failure	Autopsy
ASD-PAH3	F	32	61	2.3	CHD	NA	–	Infective endocarditis	Autopsy
ASD-PAH4	F	16	65	2.5	CHD	Negative	+	Alive (LTx)	LTx

*Known risk factors and associated conditions of PAH such as appetite suppressants, connective tissue diseases, human immunodeficiency virus infection, portal hypertension, congenital heart diseases, schistosomiasis, and chronic hemolytic anemia.[23]

ANA, indicates anti-nuclear antibody; ASD-PAH, pulmonary arterial hypertension associated with atrial septal defect; CHD, congenital heart diseases; CI, cardiac index; IPAH idiopathic pulmonary arterial hypertension; iv PGI_2, intravenous prostacyclin treatment; LTx, lung transplantation; mPAP, mean pulmonary arterial pressure; NA, not available.

Dako) for macrophages, CD45RO (1:100, Dako) for T lymphocytes, α-smooth muscle actin (α-SMA, 1:100, Dako) for smooth muscle cells, and Ki-67 (1:150, Dako) for evaluation of proliferation activity were incubated for 60 min. The EnVision+ System (Dako) was employed for visualization. Immunohistochemistry for CD44 was evaluated as the ratio of the number of plexiform lesions including CD44-positive cells per total number of lesions in each case. To identify cell types corresponding to CD44-positive elements, we proceeded with a sequence of immunohistochemical reactions using the combination of CD44 antibody and one of the following antibodies; CD31, CD68, CD45RO, or α-SMA, in the same section.

Statistical analysis

Fisher's protected least significant difference (PLSD) test and Mann–Whitney *U*-test were used to compare the IPAH, ASD-PAH, and control groups. Mean values (±SEM) are presented in the text.

RESULTS

Detection of plexiform lesions in lung tissue

The histology of lung tissue from the IPAH and ASD-PAH patients showed similar pulmonary artery remodeling, consisting of medial hypertrophy, intimal fibrosis, and plexiform lesions. We found 83 plexiform lesions, which were composed of 52 lesions from the IPAH group and 31 lesions from the ASD-PAH group (Table 2). From three to 12 plexiform lesions in the lung sections were detected per patient. On the other hand, control lung sections were confirmed to show no evidence of either pulmonary artery remodeling or interstitial inflammation.

Table 2 Distribution of CD44-positive plexiform lesions in the lung specimens of IPAH and ASD-PAH patients

Patients	Number of plexiform lesions studied (A)	Number of CD44 positive lesions (B)	B/A (%)
IPAH1	6	5	83.3
IPAH2	6	6	100
IPAH3	6	6	100
IPAH4	7	5	71.4
IPAH5	8	8	100
IPAH6	12	12	100
IPAH7	7	7	100
ASD-PAH1	12	0	0
ASD-PAH2	5	0	0
ASD-PAH3	11	0	0
ASD-PAH4	3	0	0

ASD-PAH, indicates pulmonary arterial hypertension associated with atrial septal defect; IPAH, idiopathic pulmonary arterial hypertension.

Immunohistochemical expression and localization of CD44 protein in plexiform lesions

Immunohistochemistry was performed with lung tissue from IPAH and ASD-PAH patients to determine the presence of CD44 in plexiform lesions. Staining for CD44 was detected in 49 out of 52 plexiform lesions (93%) from all patients with IPAH, ranging from 71.4 to 100% of lesions per patient, whereas plexiform lesions from ASD-PAH group did not show CD44 positivity (Table 2). In the IPAH group, the CD44-positive cells were apparently localized along the surface of thin-walled microvessels within plexiform lesions and infiltrating mononuclear cells in and around the lesions (Fig. 1a,b). Alpha-SMA-positive smooth muscle cells did not show CD44 positivity in the plexiform lesions (Data not shown). Some endothelial cells of constrictive lesions giving rise to plexiform lesions also showed CD44 positivity. Otherwise, CD44 was not expressed in vascular lesions apart from plexiform lesions, in spite of their constrictive vascular lesions (Fig. 1c,d). Immunohistochemistry was also performed with control lungs to examine whether CD44 was expressed in normal pulmonary arteries without remodeling. CD44 immunostaining was not observed in any vascular cell components of normal pulmonary arteries although a small number of alveolar macrophages were stained. Double-labeling immunohistochemistry revealed CD44-positive cells mainly corresponded to CD31-positive cells and CD45RO-positive cells, indicating that they were endothelial cells and activated T-cells, respectively (Fig. 2a,b). Serial sections of the plexiform lesions from IPAH and ASD-PAH revealed that a vast majority of CD44-positive cells (Fig. 3a) in IPAH plexiform lesion were CD45RO-positive T cells (Fig. 3b), not CD68-positive macrophages (Fig. 3c). Macrophages were also infiltrated in ASD-PAH (Fig. 3f) plexiform lesions. However, lack of immune-reaction against CD44 was apparent (Fig. 3d) and few T cells were identified (Fig. 3e) in the ASD-PAH plexiform lesion.

Inflammatory cell infiltration surrounding plexiform lesions in IPAH

In order to evaluate CD44 expression in IPAH but not in ASD-PAH, the numbers of CD45RO-positive T cell and CD68-positive macrophage inflammation per plexiform lesion for IPAH and ASD-PAH group and per normal small pulmonary artery for control group were counted. Consecutive sections showed that CD45RO-positive cells surrounding plexiform lesions were significantly increased in IPAH group compared to those in ASD-PAH (20.6 ± 1.8 versus 4.3 ± 1.3 per lesion, $P < 0.05$, Fig. 4). Plexiform lesions in ASD-PAH group included a small number of CD45RO-positive cells as

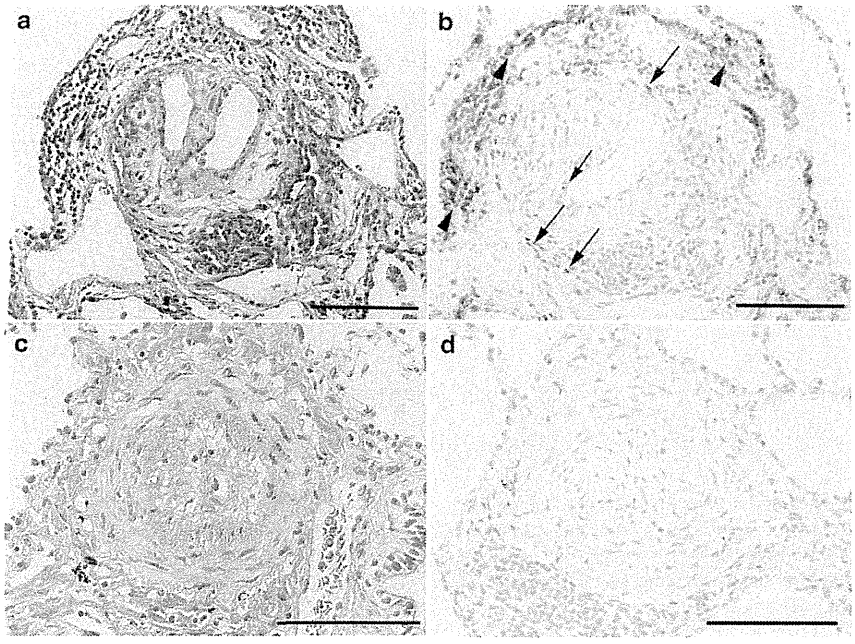

Figure 1 Hematoxylin and eosin and immunohistochemistry of CD44 in (a, b) a plexiform lesion and (c, d) a constrictive vascular lesion of a patient with idiopathic pulmonary arterial hypertension were shown. CD44 was clearly positive on the surface of thin-walled microvessels within the plexiform lesion (b; arrows) and perivascular infiltrating round cells (b; arrowheads). CD44 was not expressed in a constrictive lesion apart from plexiform lesions (d). (a-d; Scale bar 50 μm)

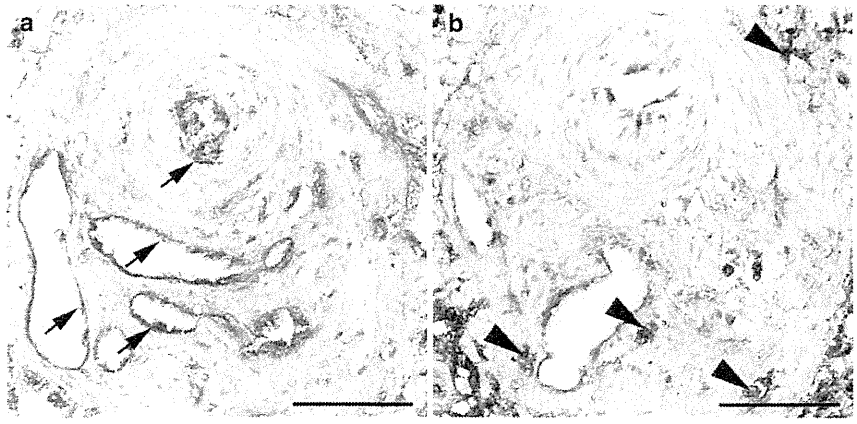

Figure 2 Double-labeling immunohistochemistry demonstrated presence of both CD44 (brown) and CD31 (red) positive cells (a; arrows) in a plexiform lesion obtained from a patient with idiopathic pulmonary arterial hypertension. Double-labeling cells of CD44 (brown) and CD45RO (red) were also evident in the same lesion (b; arrowheads). (a, b; Scale bar 50 μm)

little as control group (4.9 ± 2.4 per artery, NS). In contrast, the number of CD68-positive macrophage was similar in the IPAH group compared to those in the ASD-PAH group (10.9 ± 2.0 versus 17.5 ± 2.6 per lesion, $P = 0.50$). A few CD68-positive macrophages were observed in small pulmonary arteries obtained from normal lung tissue (0.8 ± 2.0 per artery).

Proliferation activity of endothelial cell in plexiform lesions

In order to evaluate the proliferative activity for endothelial cell of plexiform lesions in IPAH group and ASD-PAH group, immunohistochemistry for Ki-67 was examined. The number of Ki-67-positive cells in the plexiform lesions of IPAH patients was significantly higher, compared to those of the ASD-PAH group (4.2 ± 1.0 versus 0.8 ± 0.5 per lesion, $P < 0.05$, Fig. 5).

DISCUSSION

The present study demonstrated for the first time that CD44 protein was frequently expressed in plexiform lesions in the lungs from patients with IPAH and mainly localized in the endothelial cells composing microvessels of the lesions and surrounding T cells. In addition, the plexiform lesions of the IPAH group were accompanied by significant T cell infiltration. In contrast, the plexiform lesions in patients with ASD-PAH did not show detectable CD44 nor significant T cell infiltrates. These findings suggest that CD44 plays some role in the development of plexiform lesions, particularly in IPAH, presumably associated with persistent T cell inflammation.

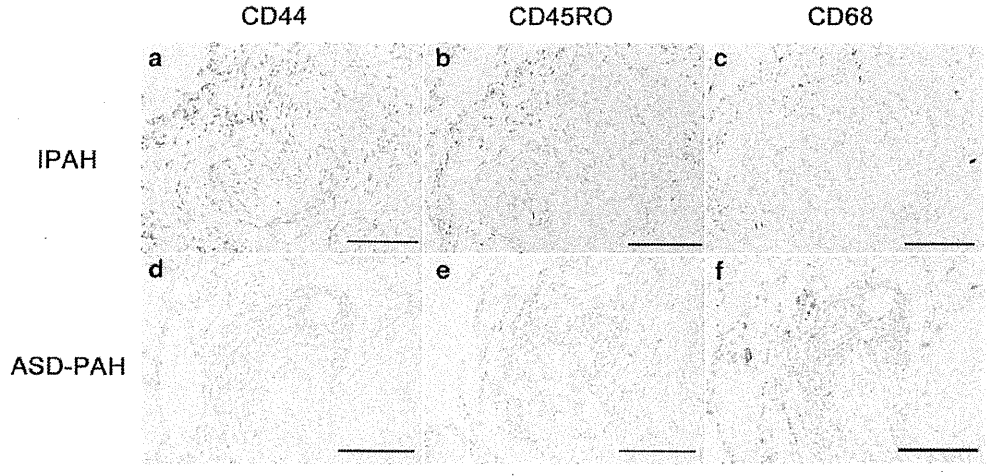

Figure 3 Immunohistochemistry of CD44, CD45RO and CD68 in the serial sections of plexiform lesion from patients with idiopathic pulmonary arterial hypertension (IPAH) and pulmonary artery hypertension associated with atrial septal defect (ASD-PAH) were shown. A vast majority of (a) CD44-positive cells in IPAH plexiform lesion were (b) T cells, not (c) CD68-positive macrophages. Macrophages were also infiltrated in (f) ASD-PAH plexiform lesions, whereas (d) lack of immune-reaction against CD44 was apparent and few T cells were identified (e) in the ASD-PAH plexiform lesion. (a-f; Scale bar 50 μm)

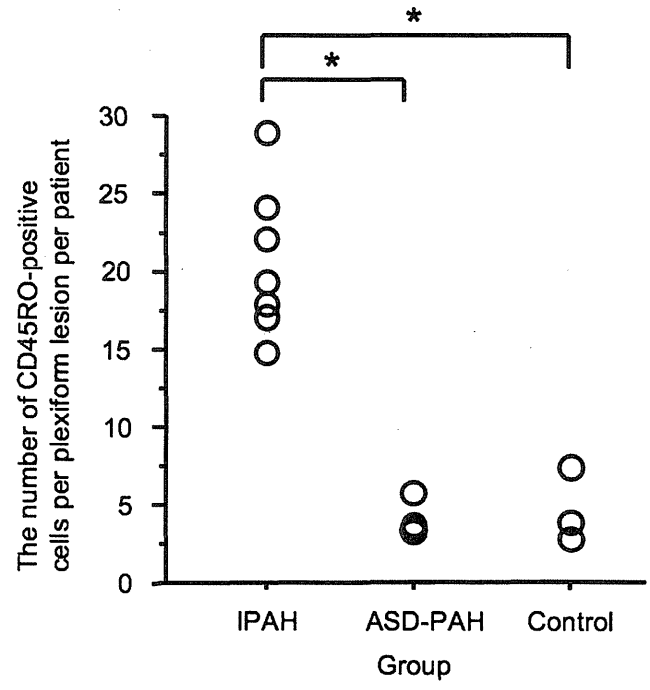

Figure 4 The chart showed the mean number of CD45RO-positive T cells per plexiform lesion in patients with idiopathic pulmonary artery hypertension (IPAH), pulmonary artery hypertension associated with atrial septal defect (ASD-PAH), and per normal small pulmonary artery in control group. Number of CD45RO-positive T cells was significantly higher in and around plexiform lesions of patients with IPAH, compared to that of ASD-PAH and normal small pulmonary arteries. (*$P < 0.01$)

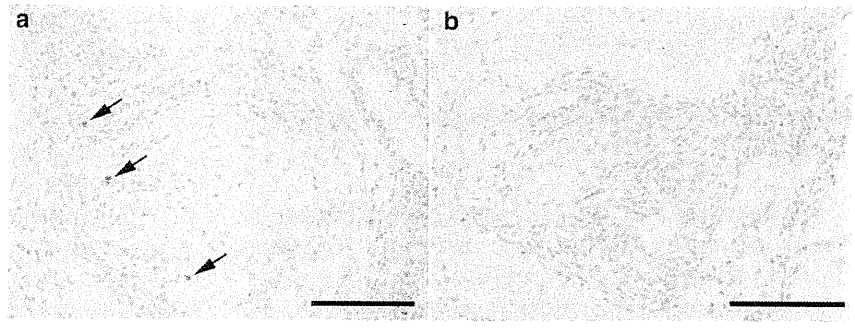

Figure 5 Immunohistochemistry of Ki-67 in plexiform lesions of a patient with idiopathic pulmonary arterial hypertension (IPAH) and pulmonary arterial hypertension associated with atrial septal defect (ASD-PAH) was shown. A certain subset of endothelial cells of IPAH plexifom lesion was apparently positive for Ki-67 immunohistochemistry (a; arrows), whereas most of the endothelial cells in the ASD-PAH plexiform lesions lacked immunoreactivity against Ki-67 (b). (a, b; Scale bar 50 μm)

CD44 is involved in cell-cell and cell-matrix interaction, and presumably maintains organ and tissue structures under normal conditions.[18] On the other hand, CD44 is shown to contribute to many pathological conditions including inflammation and autoimmune diseases, angiogenesis, atherosclerosis,[24] and malignancies and tumor metastases, via its multiple functions.

Our data implies CD44 involvement in the mechanism of persistent local inflammation observed in plexiform lesions in IPAH. Inflammatory mechanisms including inflammatory cell recruitment have become increasingly important in IPAH.[7,13–16] In the current study, CD44 expression was shown to localize in endothoelial cells and T cells in IPAH plexiform lesions. This was accompanied by significant T cell infiltration that was consistent with previous observation by other researchers.[7,16] CD44 is a homing receptor for leukocytes and required for activated T cell extravasation into an inflammatory site.[19] Increased surface levels of CD44 proteins are characteristic of T cell activation after encounter with its cognate antigen.[25] On the other hand, chronic inflammation increases CD44 expression on T cells[26] and endothelial cells express CD44 after exposure to proinflammatory cytokines such as IL-1β and TNF-α.[24] Thus, CD44 may be involved in ongoing local inflammatory processes in IPAH through T cell and endothelial cell activation and inflammatory cell recruitment.

Our data also suggests that a subset of the endothelial cells in plexiform lesions in IPAH are activated and involved in angiogenesis. CD44 is an activation marker of endothelial cells because normal endothelial cells were shown to express no or low levels of CD44, but expression is up-regulated in growing cells by activation, for example, with cytokines,[24] and by isolating and culturing of these cells or in tumor associated endothelial cells responsible for tumor angiogenesis.[22] One possible explanation for this activation is stimulation by angiogenic factors, because basic fibroblast growth factor and vascular endothelial growth factor (VEGF) are shown to upregulate CD44 expression on cultured human endothelial cells,[22] on the other hand, there is evidence of increased expression of angiogenesis-related molecules including VEGF and its receptors in endothelial cells within plexiform lesions in severe PAH.[10,12] However, other mechanisms may exist. In addition, the presence of proliferating endothelial cells by Ki-67 labeling in plexiform lesions in IPAH is in keeping with previous observation showing Ki-67-positive endothelial cells in plexiform lesions accompanied by inflammatory cells in pulmonary hypertension associated with scleroderma and human immunodeficiency virus infection.[27] Our findings support the view that endothelial cells of IPAH plexiform lesion may be prone to proliferation and these results suggest the activated stage of disease in IPAH patients.

The principle ligand of CD44 is HA.[17] Recently, two groups independently reported data suggesting an involvement of HA in the pathogenesis of IPAH. Aytekin *et al.* showed patients with IPAH had higher circulating levels of HA[28] and Papakonstantinou *et al.* showed a significant increase in HA content in IPAH lungs compared with donor.[29] Both groups showed intense HA staining in and around remodeled vessels and indicated increased HA production in pulmonary artery smooth muscle cells (PASMC) derived from IPAH lungs. In addition, PASMCs from IPAH had increased binding of mononuclear cells compared with controls.[28] These data suggests a role for HA in remodeling and inflammation via enhanced HA production in PASMC in IPAH. Because CD44 is the principle receptor for HA and its interaction regulates important processes such as cell migration, proliferation, leukocyte trafficking and activation, it is plausible that HA effects on remodeling and inflammation are mediated, at least in part, by CD44. Interestingly, data by the latter group also included significantly increased CD44 mRNA expression measured by quantitative RT-PCR in IPAH lungs compared with donor lungs,[29] in keeping with our data of increased immunohistochemical expression of CD44 in IPAH.

The intriguing finding in this study was that the plexiform lesions from the patients with ASD-PAH lacked immunohistochemical CD44 expression and were less inflamed with regard to T cells despite the similarity of morphological and hemodynamic features to IPAH. Although a relatively large number of plexiform lesions was examined, because of the limited number of patients with ASD-PAH available, the further investigation is needed to confirm the lack of CD44 in this group. However, the finding implies the difference in CD44 expression may result from different underlying pathogeneses. In fact, researchers have pointed out some distinctions between plexiform lesions in IPAH and those in congenital left-to-right shunts including ASD-PAH.[5] Importantly, in IPAH, the endothelial cell proliferation in plexiform lesions was found to be mainly monoclonal, whereas in secondary PH associated with congenital heart disease with left-to-right shunts and CREST syndrome, it was polyclonal, suggesting underlying pathogenetic mechanisms are distinct.[11] That is, monoclonal endothelial cell proliferation in IPAH is autonomous growth, more closely related to cancer cells, on the other hand, polyclonal proliferation in secondary PH is as a response to exogenous stimuli like high shear stress.[11] CD44 may be associated with abnormalities causing monoclonal expansion in IPAH, though the mechanism is yet to be determined. It will be of interest to expand our investigation to plexiform lesions in other PAH associated with underlying diseases.

Lastly, whether the IPAH patients in this study carried the BMPR2 mutation was not determined since the genetic test was not available. However the presence of inflammation would not be necessarily inconsistent with the mutation with regard to the development of the disease, because inflammatory mechanisms are now thought to be an important factor in subjects with the mutations. Heterozygous BMPR2 mutant mice develop pulmonary hypertension not spontaneously but

under inflammatory stress.[30] In addition, a complete negative feedback loop between IL-6 and BMP both *in vitro* and *in vivo* have been shown, suggesting that an important consequence of BMPR2 mutations may be poor regulation of cytokines and thus vulnerability to an inflammatory second hit.[31]

In conclusion, this study provides novel observation on the expression of CD44 predominantly in endothelial cells composing plexiform lesions and surrounding T cells in patients with IPAH, not in ASD-PAH, accompanied by significant T cell infiltration. These data suggest involvement of CD44 in the pathogenesis of IPAH, presumably associated with endothelial cell activation and persistent local inflammation.

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ORIGINAL ARTICLE
Pulmonary Circulation

Safety and Efficacy of Epoprostenol Therapy in Pulmonary Veno-Occlusive Disease and Pulmonary Capillary Hemangiomatosis

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Background: Pulmonary veno-occlusive disease (PVOD) and pulmonary capillary hemangiomatosis (PCH) are rare causes of pulmonary hypertension. There is no proven medical therapy to treat these diseases, and lung transplantation is thought to be the only cure. Administration of vasodilators including epoprostenol sometimes causes massive pulmonary edema and could be fatal in these patients.

Methods and Results: Eight patients were treated with epoprostenol for 387.3±116.3 days (range, 102–1,063 days), who were finally diagnosed with PVOD or PCH by pathological examination. The maximum dose of epoprostenol given was 55.3±10.7 ng·kg^{-1}·min^{-1} (range, 21.0–110.5 ng·kg^{-1}·min^{-1}). With careful management, epoprostenol therapy significantly improved the 6-min walk distance (97.5±39.2 to 329.4±34.6 m, P<0.001) and plasma brain natriuretic peptide levels (381.3±136.8 to 55.2±14.4 pg/ml, P<0.05). The cardiac index significantly increased from 2.1±0.1 to 2.9±0.3 L·min^{-1}·m^{-2} (P<0.05). However, pulmonary artery pressure and pulmonary vascular resistance were not significantly reduced. For 4 patients, epoprostenol therapy acted as a bridge to lung transplantation. For the other patients who had no chance to undergo lung transplantation, epoprostenol therapy was applied for 528.0±216.6 days and the maximum dose was 63.9±19.0 ng·kg^{-1}·min^{-1}.

Conclusions: This study data suggest that cautious application of epoprostenol can be considered as a therapeutic option in patients with PVOD and PCH. (*Circ J* 2012; **76:** 1729–1736)

Key Words: Epoprostenol; Pulmonary capillary hemangiomatosis; Pulmonary hypertension; Pulmonary veno-occlusive disease

Pulmonary veno-occlusive disease (PVOD) and pulmonary capillary hemangiomatosis (PCH) are rare causes of pulmonary hypertension, and their categories have been changed at every World Symposium on Pulmonary Hypertension.[1,2] The latest clinical classification of pulmonary hypertension categorized these diseases as Group 1' considering the similarity of risk factors and the genetic mutations in idiopathic pulmonary arterial hypertension (IPAH).[3,4] Continuous intravenous infusion of epoprostenol decreases pulmonary vascular resistance and improves the prognosis of IPAH,[5,6] and it has become a standard therapy for IPAH. However, the indication of epoprostenol for other subgroups of pulmonary hypertension including PVOD and PCH is controversial. A few patients with PVOD have been reported to show amelioration by application of epoprostenol.[7,8] In contrast, other reports have warned that epoprostenol precipitates severe pulmonary edema in patients with PVOD or PCH,[9,10] which never occurs in patients with IPAH. This is why epoprostenol is not widely accepted as a standard therapy for PVOD and PCH.

Montani et al reported the possible efficacy of epoprostenol for PVOD as a bridge to lung transplantation.[11] They successfully treated 12 patients (10 patients with PVOD proven by pathological studies and 2 patients with a clinical diagnosis of PVOD) for 210 days with a maximal dose of 13 ng·kg^{-1}·min^{-1} of epoprostenol. This was the first report to show the clinical application of epoprostenol therapy in a series of patients with PVOD. However, no reports have described the successful

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Table 1. Baseline Data, Histological Diagnosis and Outcome

Patient no.	Age (years)	Sex	WHO FC	Mean PAP (mmHg)	%DLco (%)	Histological diagnosis	Outcome
1	42	M	III	39	24	PVOD	Death
2	26	M	IV	60	31	PVOD	Death
3	29	M	IV	114	NA	PVOD	Death
4	11	M	IV	52	64	PCH	Death
5	25	F	IV	55	36	PCH	LDLLT
6	28	F	III	65	81	PVOD	LDLLT
7	16	F	III	63	61	PVOD	LDLLT
8	32	F	III	44	23	PVOD	LDLLT

Age, age at diagnosis; WHO FC, World Health Organization classification of functional status of patients with pulmonary hypertension; PAP, pulmonary artery pressure; %DLco, diffusion capacity of the lung for carbon monoxide expressed as % predicted; M, male; F, female; PVOD, pulmonary veno-occlusive disease; PCH, pulmonary capillary hemangiomatosis; LDLLT, living-donor lobar lung transplantation.

application of epoprostenol for PCH. We report on 8 patients (6 patients with PVOD and 2 with PCH) whose diagnoses were confirmed by pathological examination, and who were treated with a higher dose of epoprostenol and for a longer period than previously reported. With great caution, epoprostenol was safely applied and improved the clinical status in all patients. Careful application of long-term epoprostenol therapy appears to be a safe option and results in a favorable therapeutic outcome in patients with PVOD and PCH.

Methods

We treated patients with pulmonary hypertension with epoprostenol at 2 institutions (Okayama University Hospital and National Hospital Organization Okayama Medical Center, Okayama, Japan) between April 1999 and April 2010. Diagnosis of pulmonary hypertension was made according to a standard diagnostic algorithm including physical examination, chest radiograph, blood tests including screening for the cause of secondary pulmonary hypertension, pulmonary function testing, transthoracic Doppler echocardiography, and right heart catheterization.[12]

Eight patients had the clinical diagnosis of pulmonary hypertension, which was finally determined to be PVOD or PCH, in this study period. We performed a standardized chart review from the medical records to extract clinical data from them retrospectively. We compared clinical, hemodynamic, and radiographic data before and after application of epoprostenol. Data after epoprostenol treatment were obtained at the time when patients achieved the best values for the cardiac index by right heart catheterization.

Seven patients underwent pulmonary function tests when first admitted to our hospital. Vital capacity and forced expiratory volume at 1 s were calculated by using standard formulas. Diffusion capacity of the lung for carbon monoxide (DLco) was measured by the single-breath method and expressed as %DLco (% predicted). Cardiac catheterization was routinely performed at baseline before starting epoprostenol therapy and then repeatedly after starting epoprostenol therapy according to the patients' condition. Chest radiographs were obtained from all patients at the initial visit and were repeatedly taken according to their status. All patients underwent high-resolution computed tomography (CT) of the chest to define coexisting conditions, including pulmonary venous congestion, pulmonary arterial enlargement, atelectasis, or pleural effusion.

Titration of Epoprostenol Therapy

Epoprostenol therapy was initiated at a dose of 0.25–0.5 ng·kg^{-1}·min^{-1}, and the dose was gradually titrated upward in increments of 0.5–1.0 ng·kg^{-1}·min^{-1}, based on adverse effects and tolerance. When the cardiac index was below 2.0 L·min^{-1}·m^{-2}, continuous intravenous catecholamines were added to epoprostenol therapy. On adjusting the dose of epoprostenol, we paid careful attention to hypotension and signs of deterioration of heart failure and pulmonary edema. When the patients' chest radiographs showed deterioration, we stopped increasing the dose of epoprostenol and added diuretics or intravenous infusion of catecholamines, depending on the severity of pulmonary edema. After improvement, titration of the dose of epoprostenol was resumed.

Pathological Examination

No open or thoracoscopic lung biopsy was performed in any of the patients, because all patients were severely ill and they were considered intolerable to a lung biopsy. Lung specimens were obtained by living-donor lobar lung transplantation (LDLLT) or autopsy. Lung tissue was fixed in 10% formalin. Histological sections were stained with hematoxylin and eosin stain and elastica-Masson's trichrome stain.

Statistical Analysis

Results are reported as mean±standard error of the mean. Differences between groups in variables measured at baseline and after epoprostenol therapy were tested by the paired t-test. Differences were considered statistically significant at a P value of <0.05.

Results

Baseline Data, Pathological Findings and Outcome

Eight patients undergoing epoprostenol therapy had the histological diagnosis of PVOD or PCH (Table 1). The patients included 4 males and 4 females with a mean age of 26.0±3.4 years at the time of diagnosis of pulmonary hypertension. At baseline, 4 patients with PVOD were in the World Health Organization (WHO) functional class III and the other 4 patients (PVOD, n=2; PCH, n=2) were in the functional class IV. All patients showed a high mean pulmonary artery pressure (PAP) and 4 patients showed a marked decrease in %DLco as low as below 40%.

Two patients (patients 4 and 5) were finally diagnosed with PCH and the other cases were diagnosed with PVOD. Representative histology is shown in Figure 1. In all cases, foci of

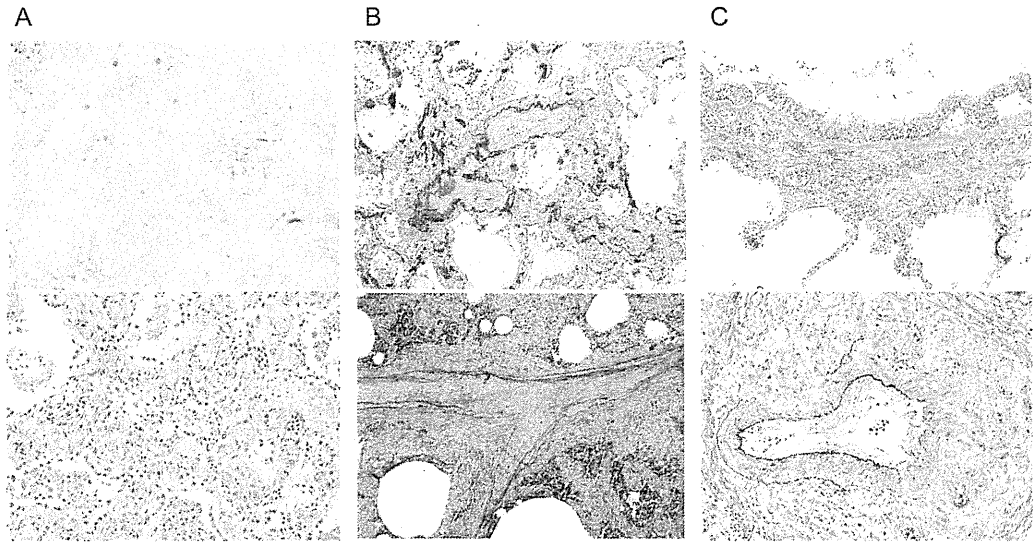

Figure 1. Pathological findings of lung specimens. (A) Specimens of pulmonary veno-occlusive disease (PVOD) show centrilobular congestion at low magnification (Upper panel) and characteristic alveolar capillaries at a higher magnification (Lower panel). These foci are seen in both PVOD and pulmonary capillary hemangiomatosis (PCH) (hematoxylin and eosin stain). (B) Venous vessel walls are thickened by intimal fibrous proliferation. Markedly stenosed (Upper panel) and completely obliterated (Lower panel) veins can be seen in PVOD (elastica-Masson's trichrome stain). (C) Proliferating capillaries are shown in the walls of bronchi (Upper panel) and arteries (Lower panel) in PCH (elastica-Masson's trichrome stain).

Table 2. Clinical and Hemodynamic Data Before and After Epoprostenol Therapy

	Baseline	After epoprostenol therapy	P value
WHO FC (n)			
II	0	5	
III	4	3	
IV	4	0	
6MWD (m)	97.5±39.2	329.4±34.6	<0.001
BNP (pg/ml)	381.3±136.8	55.2±14.4	<0.05
Hemodynamics			
Systolic PAP (mmHg)	89.4±11.0	90.9±4.9	NS
Diastolic PAP (mmHg)	44.1±7.2	43.4±4.0	NS
Mean PAP (mmHg)	61.5±8.1	61.5±3.9	NS
PCWP (mmHg)	7.0±1.3	11.8±3.6	NS
RAP (mmHg)	6.9±2.2	7.6±1.5	NS
SvO_2 (%)	59.6±5.3	64.9±4.8	NS
CI ($L \cdot min^{-1} \cdot m^{-2}$)	2.1±0.1	2.9±0.3	<0.05
PVR ($dyne \cdot s \cdot cm^{-5}$)	1,449.3±194.9	1,096.3±199.5	NS
Epoprostenol therapy			
Duration (days)		164.1±79.7	
Dose ($ng \cdot kg^{-1} \cdot min^{-1}$)		24.4±5.6	
Associated therapy (n)			
Anticoagulation	8	6	
Digitalis	4	3	
Bosentan	2	2	
Sildenafil	2	2	

After epoprostenol therapy, at the time when patients achieved the best values for cardiac index; 6MWD, 6-min walk distance; BNP, plasma concentrations of brain natriuretic peptide; PCWP, pulmonary capillary wedge pressure; RAP, right atrial pressure; SvO_2, mixed venous oxygen saturation; CI, cardiac index; PVR, pulmonary vascular resistance; duration, time from initiation of epoprostenol; NS, not significant; dose, dose of epoprostenol. All other abbreviations are as per Table 1.

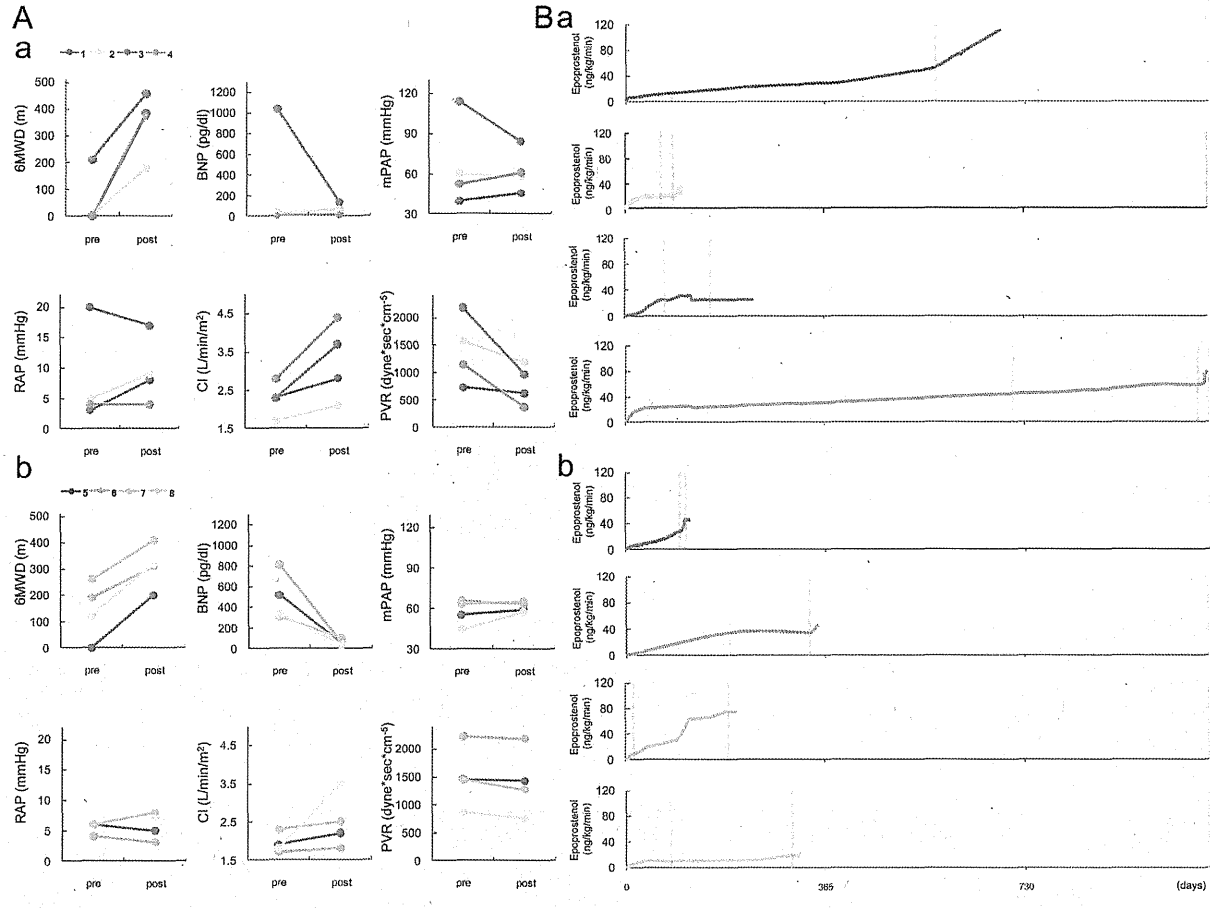

Figure 2. Changes in parameters and dose titration of epoprostenol. (A) Parameters before and after epoprostenol therapy were compared in patients without lung transplantation (a) and patients who underwent living-donor lobar lung transplantation (LDLLT) (b). Data after epoprostenol treatment were obtained at the time when patients achieved the best values for the cardiac index (CI) by right heart catheterization. The 6-min walk distance (6MWD), plasma concentrations of brain natriuretic peptide (BNP), and CI were significantly improved by epoprostenol therapy. mPAP, mean pulmonary artery pressure; RAP, right atrial pressure; PVR, pulmonary vascular resistance. (B) Dose titration of epoprostenol of each patient is shown (patients without lung transplantation (a) and patients who underwent LDLLT (b)). Red dotted lines indicate the time when patients achieved the best values for CI. Gray dotted lines indicate the time when patients showed deterioration.

Table 3. Epoprostenol Therapy and Associated Therapy

Patient no.	After epoprostenol therapy				Final	
	Time from initiation (days)	Dose ($ng \cdot kg^{-1} \cdot min^{-1}$)	Bosentan (mg/day)	Sildenafil (mg/day)	Time from initiation (days)	Dose ($ng \cdot kg^{-1} \cdot min^{-1}$)
1	82	12.5	–	–	685	110.5
2	66	15.0	–	–	102	33.7
3	70	24.9	–	60	234	32.0
4	708	46.3	–	–	1,063	79.2
Mean of patients 1–4	231.5±158.9	24.7±7.7			528.0±216.6	63.9±19.0
5	98	45.0	–	–	115	46.0
6	193	34.9	–	–	351	45.4
7	14	7.5	125	40	202	75.2
8	82	9.0	250	–	318	21.0
Mean of patients 5–8	96.8±36.9	24.1±9.4			246.5±54.2	46.7±11.1

After epoprostenol therapy, at the time when patients achieved the best values for cardiac index; final, at the time of lung transplantation or death; time from initiation, time from initiation of epoprostenol therapy; dose, dose of epoprostenol.

centrilobular congestion were observed at low magnification, and characteristic dilatation of alveolar capillaries was observed at a higher magnification (Figure 1A). Hemosiderin-laden macrophages were often observed in the alveolar space. PVOD was characterized by marked stenosis and occlusion of small intrapulmonary veins (Figure 1B). Vessel walls were thickened by intimal fibrous proliferation. In patients 4 and 5, invasive proliferation of capillaries were also observed in the walls of bronchi and arteries, leading to the diagnosis of PCH (Figure 1C). These capillaries were engorged and tortuous.

Four patients successfully underwent LDLLT and the remaining 4 patients had no suitable living donors of the lung and finally died while awaiting cadaveric lung transplantation. The causes of death were respiratory failure or concomitant respiratory infection. No patient died from adverse effects of epoprostenol itself.

Patient Characteristics Before Epoprostenol Therapy

Patient characteristics before epoprostenol therapy are shown in Table 2. All patients were in WHO functional class III and IV. The 4 patients who were in WHO functional class IV could not walk because of severe oxygen desaturation at baseline. The other 4 patients in WHO functional class III could only walk approximately 200 m (Figure 2A). Plasma BNP levels were not always elevated. Three patients showed low BNP levels in spite of the severity of their general condition and inability to walk. For the pulmonary function test, 2 patients showed mild restrictive defects (62% and 72%), and another patient showed a mild obstructive defect (65%). Overall, lung function was within normal limits (%vital capacity: 86.4±6.3%; forced expiratory volume at 1 s: 77.4±3.1%) except for low %DLco (45.8±8.6%). All patients manifested pulmonary hypertension with a mean PAP of 61.5±8.1 mmHg on right heart catheterization. Pulmonary capillary wedge pressure and right atrial pressure were within the normal range in all patients. In 4 patients, the cardiac index was lower than 2.0 $L \cdot min^{-1} \cdot m^{-2}$.

Table 4. Radiographic Findings at Baseline and After Epoprostenol Therapy

Radiographic findings	PVOD and PCH (n=8)
Baseline	
Dilated pulmonary arteries	8
Kerley B lines	2
Interstitial infiltrates	8
Ground-glass opacities	7
Pleural effusion	2
Interlobular thickening	8
Lymphadenopathy	3
After epoprostenol therapy	
Increase in pleural effusion	3
Thickened interlobular septae	8
Deterioration of ground-glass opacities	8

Data indicates the number of patients.
PVOD, pulmonary veno-occlusive disease; PCH, pulmonary capillary hemangiomatosis.

Efficacy of Epoprostenol Therapy

Patients were cautiously treated with epoprostenol for 387.3±116.3 days (range, 102–1,063 days) (Table 3; Figure 2B). The maximum dose of epoprostenol given was 55.3±10.7 $ng \cdot kg^{-1} \cdot min^{-1}$ (range, 21.0–110.5 $ng \cdot kg^{-1} \cdot min^{-1}$). Patients who had no chance to undergo a lung transplantation had epoprostenol therapy applied for 528.0±216.6 days and the maximum dose was 63.9±19.0 $ng \cdot kg^{-1} \cdot min^{-1}$. The best value for cardiac

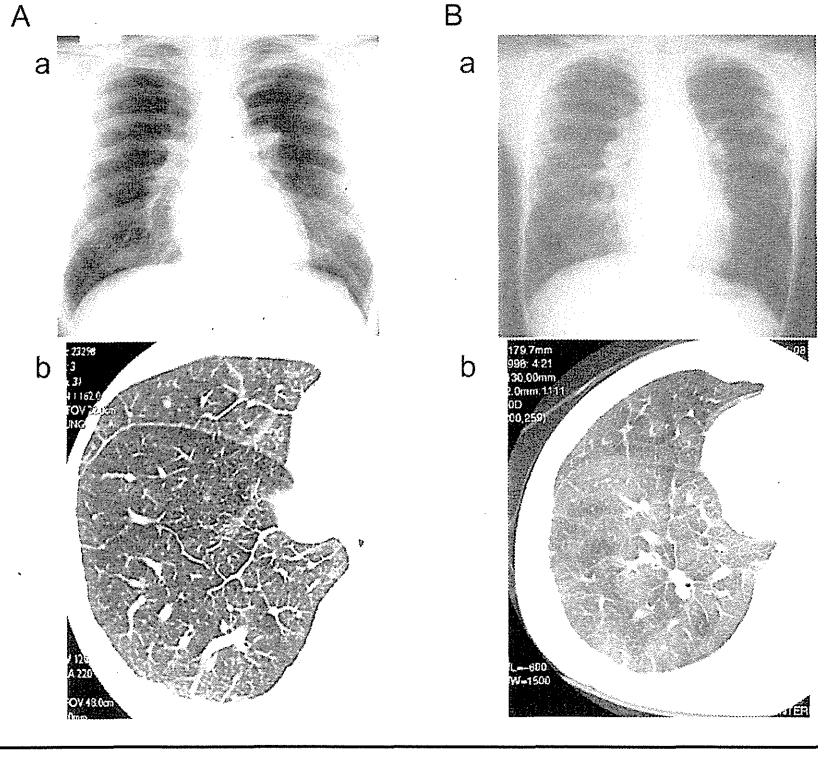

Figure 3. Representative radiographic findings. (A) Radiographic findings of pulmonary veno-occlusive disease (patient 1). (a) A chest radiograph shows a prominent main pulmonary artery and interstitial infiltrates. (b) A chest computed tomography (CT) scan of the right lobe shows multiple thickened interlobular septal lines, ground-glass opacities, and pleural effusion. (B) Radiographic findings of pulmonary capillary hemangiomatosis (patient 4). (a) A chest radiograph shows a prominent main pulmonary artery and main truncus of the right pulmonary artery. Faint nodular opacities and numerous Kerley B lines are also shown. (b) A chest CT scan shows diffuse, ill-defined, ground-glass opacities, and interlobular septal thickening is less prominent.