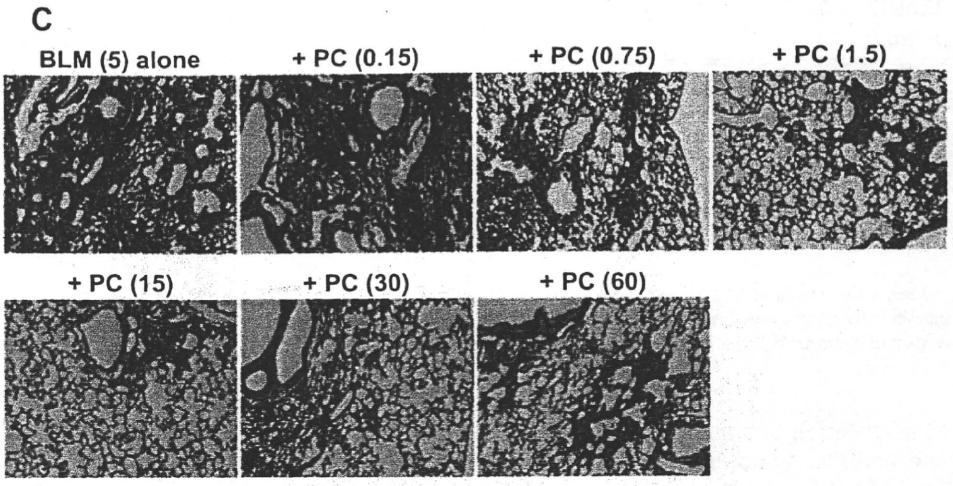
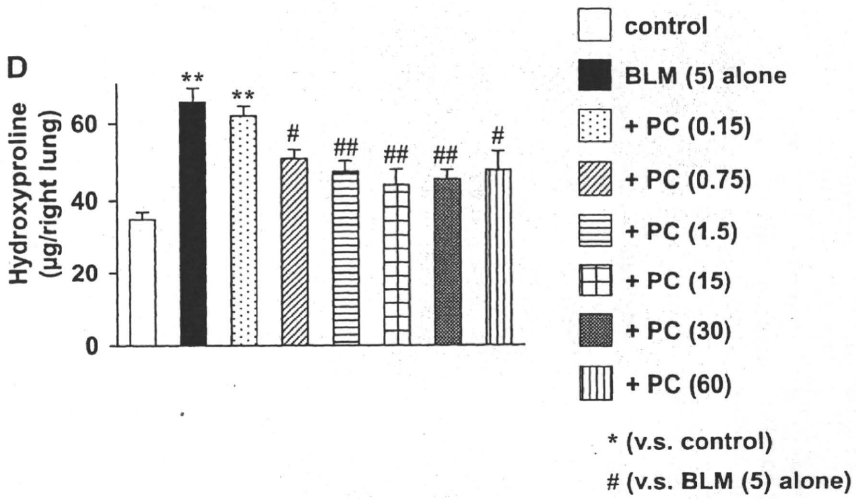


Fig. 5—*Continued*



route of administration; the intratracheal administration of higher doses of PC-SOD (30 or 60 kU/kg) showed similar ameliorative effects to those seen for lower doses (Fig. 5*A*). As shown in Fig. 5, *B–D*, the intratracheal administration of PC-SOD also suppressed bleomycin-induced pulmonary tissue damage and fibrosis. Again, the bell-shape dose-response profile was not so obvious.

As shown in Table 1, after daily intratracheal administration of PC-SOD, the pulmonary level of PC-SOD was very high compared with that seen following intravenous administration. We therefore compared the distribution of PC-SOD in lung tissue in response to intravenous and intratracheal administration using immunohistochemical analysis with antibody against human Cu/Zn-SOD. As shown in Fig. 6, SOD was detected depending on the administration of PC-SOD, showing that this antibody specifically recognizes administered PC-SOD (not endogenous mouse SOD) under the conditions used. PC-SOD was detected in tissues containing a major airway but was not as evident in regions distant from trachea after the intratracheal administration of a low dose (1.5 kU/kg) (Fig. 6). On the other hand, PC-SOD was widely detected in both regions after the intravenous administration of a high dose (30 kU/kg) (Fig. 6). No SOD staining was observed in any regions after the intravenous administration of a low dose of PC-SOD (1.5 kU/kg) (data not shown).

PC-SOD was also detected in the serum after intratracheal administration; however, the level was much lower than that measured after its intravenous administration at an equivalent dose (Table 1).

The results shown in Fig. 5 suggest that inhalation of PC-SOD may increase the QOL of patients in the clinical practice. To test this idea, bleomycin-administered mice were placed in a chamber connected to an ultrasonic nebulizer, thus exposing them to PC-SOD-containing vapor. We confirmed by HPLC analysis and measurement of SOD activity that this treatment did not affect the structure and activity of the PC-SOD (data not shown). This treatment was repeated once daily for 3 days or 14 days, and bleomycin-induced pulmonary disorders were examined. As shown in Fig. 7*A*, inhaled PC-SOD [both low dose (60 kU/chamber) and high dose (300 kU/chamber)] ameliorated the bleomycin-induced inflammatory response and suppressed the pulmonary tissue damage and fibrosis (Fig. 7, *B–D*). We also found that inhalation of an even higher dose of PC-SOD (900 kU/chamber) decreased the bleomycin-induced inflammatory response as much as its low dose (60 kU/chamber) (data not shown), suggesting that bell-

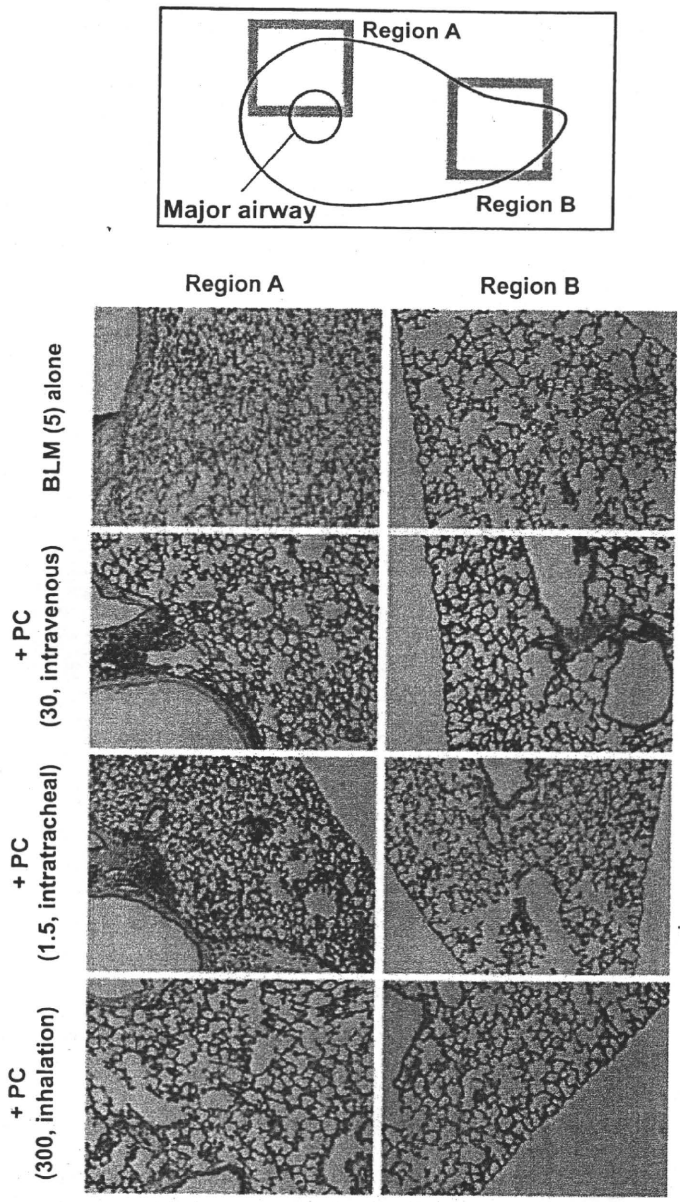


Fig. 6. Distribution of PC-SOD in the lung. Mice were treated with bleomycin, and indicated doses of PC-SOD (kU/kg or kU/chamber) were administered intravenously, intratracheally, or by inhalation once per day for 3 days. Sections of pulmonary tissue (from the 2 regions shown) were prepared 6 h after the final administration of PC-SOD (after 3 days) and subjected to immunohistochemical analysis with an antibody against human Cu/Zn-SOD. Similar results were obtained for at least 3 sections.

shaped dose-response profile did not occur with inhalation. As shown in Table 2, administration of not only a low dose (60 kU/chamber) but also a high dose (300 kU/chamber) of PC-SOD did not increase the pulmonary level of hydrogen peroxide, being different from the case of intravenous administration. We also found that inhalation of unmodified SOD did not affect the bleomycin-induced inflammatory response (Table 3). As shown in Table 1, PC-SOD was detected in the pulmonary tissue after daily sessions of inhalation. Immunohistochemical analysis revealed that inhaled PC-SOD was distributed broadly in the lung tissue (Fig. 6). Furthermore, very little PC-SOD was detected in serum following its delivery in this manner (Table 1).

DISCUSSION

Previous studies showed that intravenous administration of PC-SOD ameliorates bleomycin-induced pulmonary fibrosis; however, its molecular mechanism was not fully understood (44, 50). In these studies, a bell-shaped dose-response profile for PC-SOD was observed, but the mechanism underlying this effect was unclear. In the present study, we reproduced the results of the previous studies and examined underlying mechanisms. Furthermore, as the current clinical protocol for the administration of PC-SOD (once daily intravenous infusion for 4 wk) does not provide patients with good QOL, we attempted to find other dosing regimes in our animal model with a view to provide better clinical outcomes.

Pulmonary cell death could be a trigger of IPF and bleomycin-induced pulmonary fibrosis because it stimulates the inflammatory response and fibrosis (abnormal wound repair and remodeling) as described in the introduction. We showed that pulmonary cell death in bleomycin-treated mice was suppressed by administration of PC-SOD. We also showed that PC-SOD protected cultured lung epithelial cells from menadione-induced cell death. Furthermore, we found that PC-SOD suppresses the bleomycin-dependent increase in TGF-β1 levels in pulmonary tissue in vivo and menadione-induced production of TGF-β1 in vitro. On the other hand, PC-SOD did not affect the TGF-β1-dependent stimulation of collagen synthesis and induction of EMT. Based on these findings, we consider that PC-SOD ameliorates bleomycin-induced pulmonary fibrosis through its inhibitory effect on ROS-induced cell death and expression of TGF-β1 rather than by modulating TGF-β1-dependent cellular responses.

The bell-shaped dose-response profile of PC-SOD is of clinical concern, as this may reflect side effects of the drug. Here, however, we found that the efficacy of higher doses of PC-SOD is restored by simultaneous administration of catalase, which converts hydrogen peroxide to water and oxygen. As such, the ineffectiveness of high doses of PC-SOD on bleomycin-induced pulmonary fibrosis is likely to be caused by accumulation of hydrogen peroxide. The simultaneous administration of catalase with PC-SOD to IPF patients may therefore provide a greater therapeutic effect and lower the risk of side effects. Furthermore, the examination of catalase activity in individuals before PC-SOD administration may result in the establishment of safer treatment protocols for IPF patients.

We also found that intratracheal administration of PC-SOD significantly suppressed bleomycin-induced pulmonary fibrosis. PC-SOD was detected in the serum following this mode of administration; however, the serum level with intratracheal administration of PC-SOD (1.5 kU/kg, effective dose for bleomycin-induced pulmonary fibrosis) was much lower than that measured following the intravenous administration of PC-SOD (0.75 kU/kg, ineffective dose). Therefore, it seems that the delivery of PC-SOD directly to the lung (but not via the blood) is primarily responsible for the improved effects seen in response to its intratracheal administration. On the other hand, the pulmonary level of PC-SOD administered intravenously (1.5 kU/kg, effective dose) was much lower than that obtained with intratracheal administration (0.15 kU/kg, ineffective

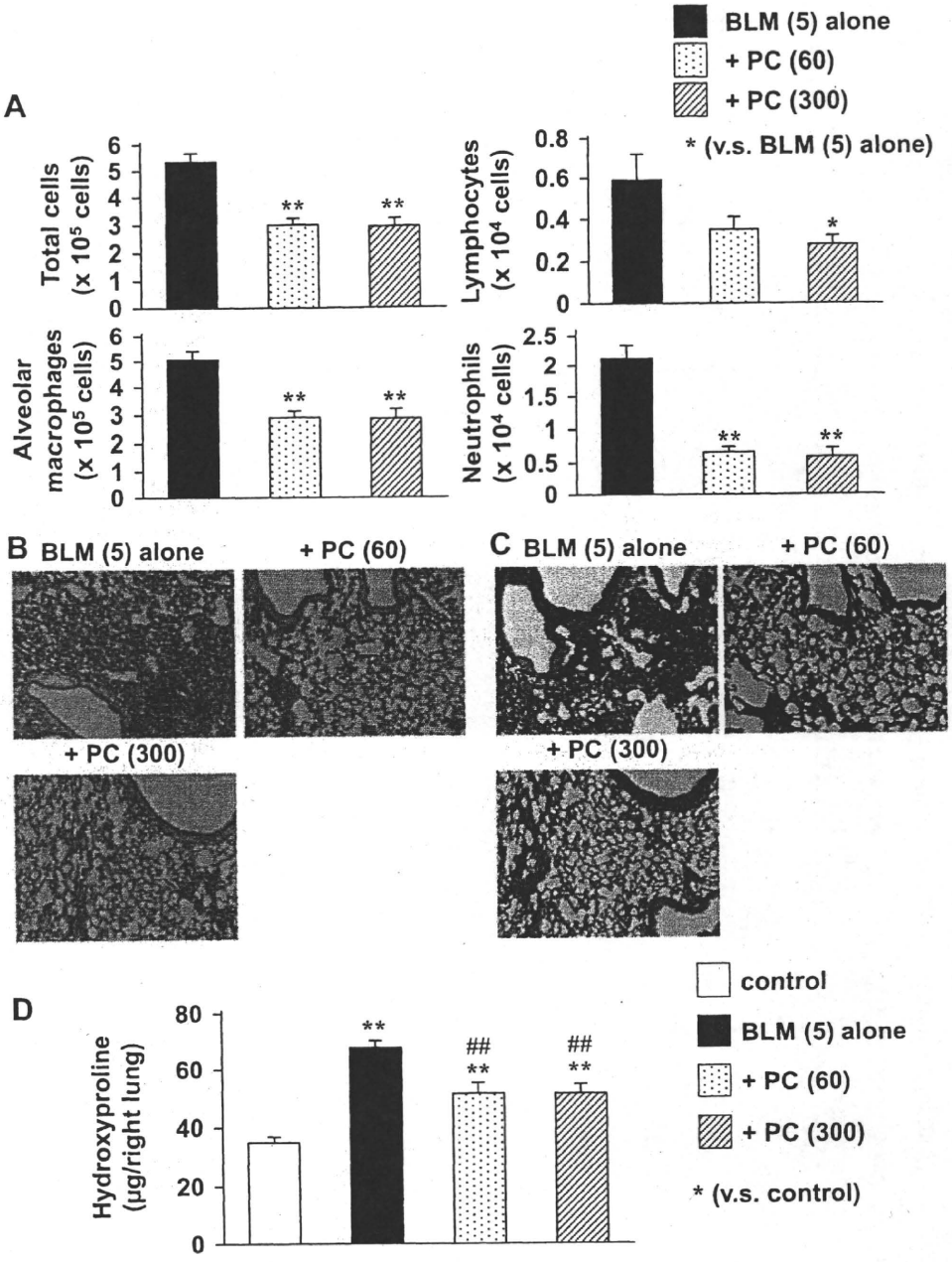


Fig. 7. Effect of inhalation of PC-SOD on bleomycin-induced inflammatory response and pulmonary fibrosis. Mice were treated with bleomycin, and the inflammatory response (*A*) and pulmonary fibrosis (*B–D*) were assessed as described in the legends of Figs. 1 and 2. The indicated doses of PC-SOD (kU/kg) were inhaled once per day for 3 days (*A*) or 14 days (*B–D*). Similar results were obtained for at least 3 sections (*B* and *C*). Values are means ± SE. *$P < 0.05$; ** or ##$P < 0.01$.

dose). This may due to the localization of intratracheally administered PC-SOD close to the trachea rather than regions distant from there. Therefore, it seems that PC-SOD should be delivered in a broad manner to the lung to suppress bleomycin-induced pulmonary fibrosis. It should also be noted that the bell-shaped dose-response profile of PC-SOD was not observed (up to 60 kU/kg) with the intratracheal mode of administration.

Table 3. *Effect of inhalation of U-SOD on bleomycin-induced inflammatory response*

U-SOD, Inhalation, kU/Chamber	Total Cells (×10⁵ Cells)	Alveolar Macrophages (×10⁵ Cells)	Lymphocytes (×10⁴ Cells)	Neutrophils (×10⁴ Cells)
Control	5.1 ± 0.28	4.8 ± 0.26	0.5 ± 0.01	1.7 ± 0.15
60	5.1 ± 0.14	4.8 ± 0.14	0.5 ± 0.07	1.6 ± 0.08
300	4.6 ± 0.23	4.4 ± 0.25	0.5 ± 0.08	1.5 ± 0.22

Mice were treated with bleomycin, and the inflammatory response was assessed as described in Fig. legend 1. Indicated doses of unmodified SOD (U-SOD; kU/chamber) were inhaled once per day for 3 days. Values are means ± SE.

We also found that inhalation of PC-SOD ameliorated bleomycin-induced pulmonary fibrosis. This finding is very important because if this mode of administration of PC-SOD can be applied clinically, it should greatly improve the QOL of patients treated with the drug. The lack of a bell-shaped dose-response profile with this route of administration is also therapeutically beneficial. The pulmonary level of PC-SOD after inhalation of PC-SOD (900 kU/chamber, effective dose) was higher than that after the intravenous administration (30 kU/kg, ineffective dose due to the bell-shaped profile). This discrepancy may be due to the difference in the local distribution of PC-SOD (for example, in the alveolar epithelia or in vessel

walls). It was recently reported that inhalation of NAC attenuates bleomycin-induced pulmonary fibrosis (15). Since NAC stimulates the conversion of hydrogen peroxide to water and oxygen (12, 27), simultaneous administration of PC-SOD and NAC by inhalation may have a synergistically therapeutic effect on bleomycin-induced pulmonary fibrosis and IPF.

A phase II clinical study has shown that intravenously administered PC-SOD (40 or 80 mg) showed therapeutic effects against IPF as judged by the serum level of markers (lactate dehydrogenase and surfactant protein A) (Azuma A, Ohta K, Sugiyama Y, Nukiwa T, Kudoh S, unpublished results). Based on results in this study, we propose that the inhalation mode for administering PC-SOD could prove beneficial for the treatment of IPF patients. This is because compared with intravenous administration, this mode of administration would cause improvement of the QOL of patients treated with the drug, equivalent efficacy (judged by immunohistochemical analysis in this study), and superior safety (due to lack of a bell-shaped dose-response profile). This mode of administration may be effective for other pulmonary diseases, such as chronic obstructive pulmonary disease and asthma, in which ROS-induced pulmonary damage also plays an important role (28, 34, 35).

GRANTS

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DISCLOSURES

No conflicts of interest are declared by the author(s).

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INTERNAL MEDICINE

□ ORIGINAL ARTICLE □

Long-Term Efficacy of Inhaled N-Acetylcysteine in Patients with Idiopathic Pulmonary Fibrosis

Masashi Bando[1], Tatsuya Hosono[1], Naoko Mato[1], Takakiyo Nakaya[1], Hideaki Yamasawa[1], Shoji Ohno[2] and Yukihiko Sugiyama[1]

Abstract

Background Inhalation of N-acetylcysteine (NAC) has been carried out in our department since 1994 for treating interstitial pneumonia such as idiopathic pulmonary fibrosis (IPF). In this study, the clinical efficacy and safety of long-term NAC inhalation monotherapy for IPF was investigated.

Methods NAC inhalation was carried out in 23 of 34 cases diagnosed as IPF by surgical lung biopsy in our department between 1994 and 2008. The treatment was continued for one year or longer in 14 cases. In these 14 cases and in 11 cases without treatment, the clinical courses, prognosis, lung function (%FVC, %DLco, and %TLC), and changes in serum markers for interstitial pneumonia (KL-6 and SP-D) were examined.

Results There were no significant differences in survival curves between the two groups. Acute exacerbation was observed in 4 of 14 cases (28.6%) receiving NAC inhalation. Compared with the results just before the beginning of NAC inhalation, Δ%FVC and Δ%DLco in the treated cases were -4.7% and -2.9% one year later and -4.0% and -5.8% two years later, respectively. In cases without treatment, Δ%FVC and Δ%DLco were -3.5% and +5.3% one year later and +0.2% and +1.0% two years later, respectively.

Conclusion Since this study is an open case-control study in a single institute and the number of cases is not large, its use in evaluating the efficacy of NAC inhalation monotherapy is limited. In addition, the role of NAC inhalation in combination with a steroid, an immunosuppressive agent, and a new anti-fibrosis drug should also be investigated.

Key words: idiopathic pulmonary fibrosis, inhalation, N-acetylcysteine (NAC)

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Introduction

Idiopathic pulmonary fibrosis (IPF) is a chronic progressive interstitial pneumonia with a poor prognosis (1). The median survival of patients with IPF is 3-5 years after the onset of symptoms (2). Since it is difficult to improve the survival time by anti-inflammatory therapy with a simple combination of a corticosteroid and an immunosuppressant such as cyclophosphamide and azathioprine (2), treatment with N-acetylcysteine (NAC), pirfenidone, and endothelin receptor antagonists have received attention as a new strategy targeting alveolar-epithelium injury and control of abnormalities of fibroblasts (3-5).

It has been proposed that a pathogenetic mechanism of IPF is repeated epithelial injury with oxidant-antioxidant imbalance (6). NAC, is a precursor to the major antioxidant glutathione, which may be reduced in the lung of patients with IPF (7, 8). A recent multicenter randomized trial, the IFIGENIA study, tested the effect of oral high-dose NAC versus placebo in patients receiving prednisone and azathioprine (3). This study demonstrated significantly better preserved vital capacity (VC) and diffusing capacity for carbon monoxide (DLco). However, a substantial number of patients dropped out of the study, and the effect on the outcome of the patients who withdrew from the study is not known. Therefore, a combination corticosteroid, azathioprine and NAC therapy in patients with IPF is not recommended.

[1]Division of Pulmonary Medicine, Department of Medicine, Jichi Medical University, Tochigi and [2]Division of Pulmonary Medicine, Department of Medicine, International University of Health and Welfare Hospital, Nasushiobara
Received for publication May 27, 2010; Accepted for publication July 22, 2010
Correspondence to Dr. Masashi Bando, bando034@jichi.ac.jp

Inhaled NAC has been used for many years as a mucolytic agent in Japan that is largely free of adverse effects. Therefore, inhalation of NAC has been employed since 1994 in our department for interstitial pneumonia such as IPF. In the present study, we evaluated the clinical effect of long-term NAC inhalation monotherapy for 1 year or longer in patients with IPF diagnosed by surgical lung biopsy. We also evaluated problems with the continuation of NAC inhalation monotherapy.

Materials and Methods

Patients

We conducted a retrospective study of 34 patients diagnosed as IPF by surgical lung biopsy in our hospital between 1994 and 2008. Histologic evidence demonstrated the characteristics of usual interstitial pneumonia (UIP) and showed the various stages of interstitial lung disease including alveolitis, fibrosis, honeycombing, and a patchy reticular pattern that was predominantly evident in the basal region of the periphery of the lung fields. Patients who had received any corticosteroids or immunosuppressant drugs prior to lung biopsy were ineligible. Patients were excluded if they had clinical or serologic evidence of collagen vascular disease, a history of exposure to known fibrogenic agents, active infection, malignancy, hypersensitive pneumonitis or acute respiratory distress syndrome (ARDS). Pulmonary function tests (percentage of predicted forced vital capacity [%FVC], percentage of predicted carbon monoxide diffusing capacity[%DLco], percentage of predicted total lung capacity[%TLC]) and blood gas analysis (PaO_2) were performed on enrollment into this study. Clinical features, changes in pulmonary function tests (ΔFVC, Δ%FVC, Δ%DLco, and Δ %TLC), changes in serum markers for interstitial pneumonia (KL-6 and SP-D), and prognoses of all patients were retrospectively evaluated. Acute exacerbation was defined using the following criteria proposed by IPF Clinical Research Network (9) [1] previous or concurrent diagnosis of IPF, [2] unexplained worsening or development of dyspnea with 30 days, [3] high-resolution computed tomography with new bilateral ground-glass abnormality and/or consolidation superimposed on a background reticular or honeycomb pattern consistent with UIP pattern, and [4] no evidence of alternative causes, including the following: left heart failure, pulmonary embolism, identifiable causes of acute lung injury.

Therapeutic regimen and evaluation

NAC inhalation monotherapy was carried out in 23 of 34 cases. The therapy was delivered via a compressor-type nebulizer (OMRON, Tokyo). Patients inhaled 352.4 mg NAC diluted with saline to a total volume of 10mL twice a day. This type of nebulizer produces aerosol particles of 1-10 μm and the 50% particle diameter is 5.21 μm. The treatment was continued for one year or longer in 14 of 23 cases. In these 14 cases and in 11 cases without treatment, the clinical features, changes in pulmonary function tests (% FVC, %DLco, and %TLC), changes in serum markers for interstitial pneumonia [KL-6 and surfactant protein-D (SP-D)], and prognoses of all patients were retrospectively evaluated. Efficacy was assessed by changes in results of pulmonary function tests from baseline to 12 months or 24 months. Serum KL-6 was measured by a sandwich electrochemiluminescence immunoassay (ECLIA) for routine laboratory use (Picolumi KL-6, Sanko, Tokyo, Japan). SP-D was measured by an enzyme-linked immunosorbent assay (ELISA) using monoclonal antibodies to human SP-D (SP-D EIA Kit, Yamasa, Tokyo, Japan). Although unavoidable administration of corticosteroids or immunosuppressive drugs for acute exacerbation or progression of the condition during the follow-up period was permitted, such treatment was prohibited in stable patients. Clinical data after the introduction of drug administration in cases with acute exacerbation, progress/deterioration, and administration of corticosteroids or immunosuppressants were excluded from analysis.

Safety was assessed by clinical symptoms (cough, dyspnea, appetite loss), drop out rate and fatal adverse events. Informed consent was obtained from all participants and the study protocol was approved by the local ethics committee. The withdrawal criteria for NAC inhalation were: withdrawal of the consent by the patient; judgment by the physician in charge to stop inhalation due to adverse events and safety reasons; and judgment of difficulty in inhalation due to acute exacerbation of IPF and aggravation from other causes.

Survival rates

The survival rate after treatment with or without NAC were compared by the log rank test and Kaplan-Meier survival curves were plotted. Survival status in August 2009 was established from clinical records.

Statistical methods

Data are presented as mean ± SD or as median (interquartile range). Differences between subjects in the two groups were examined by the Mann-Whitney U-test or the Chi-squared test. Kaplan-Meier analysis was used to compare survival between the groups. Changes in parameters are expressed as an absolute value. ANOVA was used to compare the mean changes in values from baseline to 12 months or 24 months in the two treatment groups. Significance was defined as $p<0.05$.

Results

Status of NAC inhalation

There were 14 cases who were able to continue NAC inhalation one year or longer, and the average period of NAC inhalation was 29.9 months (12-88 months). Five cases continued the treatment for two years or longer. Death was the most frequent cause for stopping NAC inhalation, followed

Table 1. Baseline Characteristics of the Study Population

	NAC inhalation group (n=14)	Non-treatment group (n=11)	p-value
Age (yrs)	62 (50-74)	63 (55-71)	
Sex (n)			
Male	9	9	
Female	5	2	
Smoking status			
Smoker	4	1	
Ex-smoker	5	7	
Never smoker	5	3	
Symptom DOE	10 (71.4%)	8 (72.7%)	
Time since DOE (months)	12 (0-48)	4.4 (0-12)	0.11
KL-6 (U/m)	2472 ± 1222	1809 ± 1053	0.19
SP-D (ng/mL)	388 ± 65.0	334 ± 114.6	0.26
LDH (IU/L)	463 ± 136	410 ± 151	0.37
FVC (L)	2.52 ± 0.67	2.72 ± 0.93	0.51
%FVC (%)	84.2 ± 14.7	85.1 ± 24.6	0.91
%DLco (%)	41.4 ± 17.1	52.5 ± 15.5	0.12
%TLC (%)	78.8 ± 11.6	91.5 ± 12.9	0.05
PaO_2 (Torr)	84.4 ± 11.7	87.0 ± 14.9	0.63

NAC = N-acetylcysteine; DOE = dyspnea on effort; FVC = forced vital capacity; %FVC = percentage of predicted forced vital capacity; %DLco = percentage of predicted carbon monoxide diffusing capacity; %TLC = percentage of predicted total lung capacity

Table 2. Clinical Course

	NAC inhalation group (n=14)	Non-treatment group (n=11)
Long-term oxygen therapy	4	0
Acute exacerbation	4	2
Period from diagnosis of IPF to AE		
Range (months)	4 - 88	2 - 11
Cases developing AE within 12 months from diagnosis (n)	3	2
Death	11	3
IPF progression	1	0
Acute exacerbation	4	2
Others	3	1
Unknown	3	0

AE = acute exacerbation

by adverse drug reactions such as sulfurous smell and cough. Of nine cases that continued NAC inhalation only for less than one year, continuing inhalation was difficult in four cases due to adverse effects such as cough and appetite loss.

Patients

Baseline characteristics of the study population are shown in Table 1. There were no differences between these two groups.

Clinical course and prognosis

In terms of the subjective symptomatic changes in 14 cases that had been able to continue NAC inhalation for more than one year, dyspnea on exertion improved in 1 and deteriorated in 4 with no change in 9.

Clinical course is shown in Table 2. Due to respiratory insufficiency, long-term oxygen therapy was introduced in four of 14 cases (28.6%) receiving NAC inhalation. During the follow-up, acute exacerbation was observed in four cases (28.6%). On the other hand, acute exacerbation was observed in two of 11 cases (18.2%) without treatment. Eleven cases receiving NAC inhalation and three without treatment died. There was no significant difference in the survival curves between the two groups, and the average survival period was 50.1 months after the beginning of NAC inhalation (Fig. 1).

Changes in respiratory function test results

Year-to-year changes in respiratory test results (ΔFVC, Δ%FVC, Δ%DLco, and Δ%TLC) in the groups with and without NAC inhalation are shown in Table 3. Δ%FVC and Δ%DLco were -4.7% and -2.9% one year later and -4.0% and -5.8% two years later, respectively, in the NAC inhalation group. In cases without treatment, Δ%FVC and Δ%DLco were -3.5% and +5.3% one year later and +0.2% and

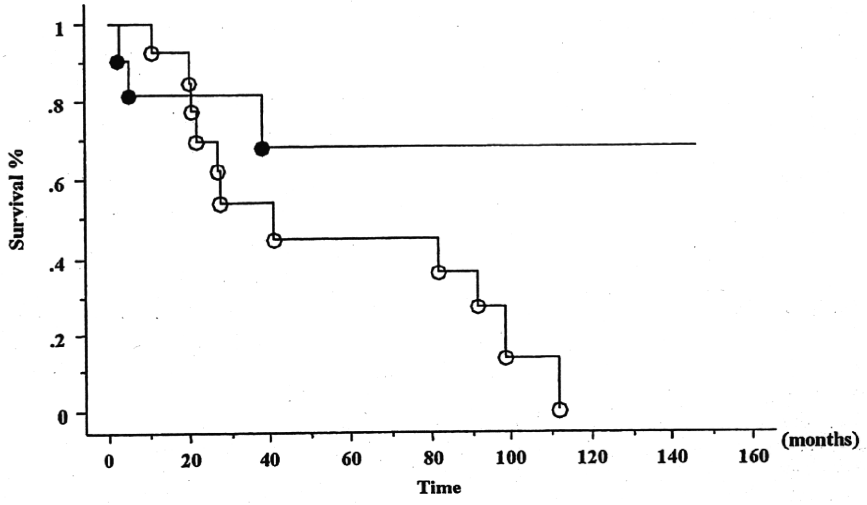

Figure 1. Survival curve. There was no significant difference in the Kaplan-Meier survival curves between the two groups. The average survival period was 50.1 months after the beginning of NAC inhalation. ○: NAC inhalation group, ●: Non-treatment group

Table 3. Year-to-Year Changes in Respiratory Test Results in the Groups with and without NAC Inhalation

	NAC inhalation group	Non-treatment group	p-value
ΔFVC (mL)			
12 months	-133.0 (-790 ~ +680 ; 6)	-166 (-330 ~ +110 ; 5)	0.89
24	-151.4 (-850 ~ +230 ; 7)	-31.7 (-310 ~ +130 ; 3)	0.60
Δ%FVC (%)			
12 months	-4.7 (-23.6 ~ +17.6 ; 6)	-3.5 (-9.7~ +6.5 ; 5)	0.86
24	-4.0 (-28.2 ~ +7.0 ; 7)	+0.2 (-9.1~ +6.7 ; 3)	0.54
Δ%DLco (%)			
12 months	-2.9 (-14.6 ~ +7.4 ; 6)	+5.3 (-6.5 ~ +19.3 ; 4)	0.22
24	-5.8 (-42.8 ~ +13.2 ; 7)	+1.0 (-1.9 ~ +2.7 ; 3)	0.53
Δ%TLC (%)			
12 months	-5.2 (-20.1 ~ +10.7 ; 6)	-2.4 (-6.1 ~ +4.8 ; 4)	0.62
24	-3.6 (-11.7 ~ +1.7 ; 6)	+0.6 (-2.0 ~ +4.4 ; 3)	0.21
	mean (range; n)	mean (range; n)	

ΔFVC = changes in forced vital capacity; Δ%FVC = changes in percentage of predicted forced vital capacity; Δ%DLco = changes in percentage of predicted carbon monoxide diffusing capacity; Δ%TLC = changes in percentage of predicted total lung capacity

+1.0% two years later, respectively, and there were no significant differences between the two groups. Distribution of Δ%FVC from the beginning of observation is shown in Fig. 2. In the NAC inhalation group, year-to-year changes were diverse and an improvement of 10% or more in %FVC one year later was observed in 1 of 6 cases. However, deterioration of 10% or more was found in one case receiving only NAC inhalation.

Changes in serum markers for interstitial pneumonia

In one case receiving NAC inhalation, KL-6 improved remarkably after the treatment was started, but changes were inconsistent in other cases and there were no significant differences in year-to-year changes of KL-6 or SP-D between the two groups (Fig. 3).

Comparison of Clinical Background between 4 Acute IPF Exacerbation and 10 Non-exacerbation Cases

There were no significant differences in clinical background between the cases who experienced exacerbations and those who did not. However, in three patients whose pulmonary function parameters were able to be sequentially measured, pulmonary function worsened before developing acute exacerbation (date not shown).

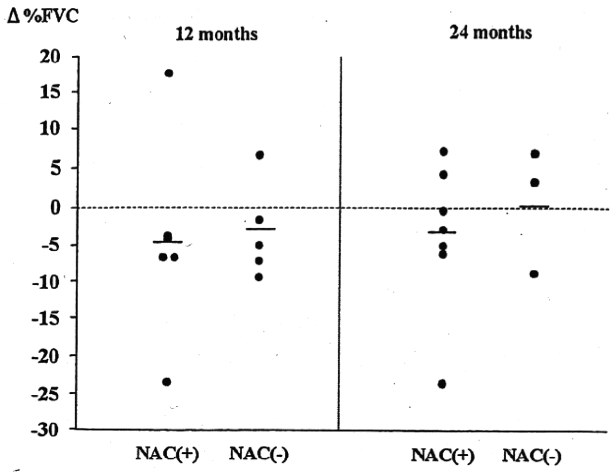

Figure 2. Distribution of Δ%FVC from the beginning of observation. In the NAC inhalation group, year-to-year changes were diverse and an improvement of 10% or more in %FVC one year later was observed in 1 of 6 cases.

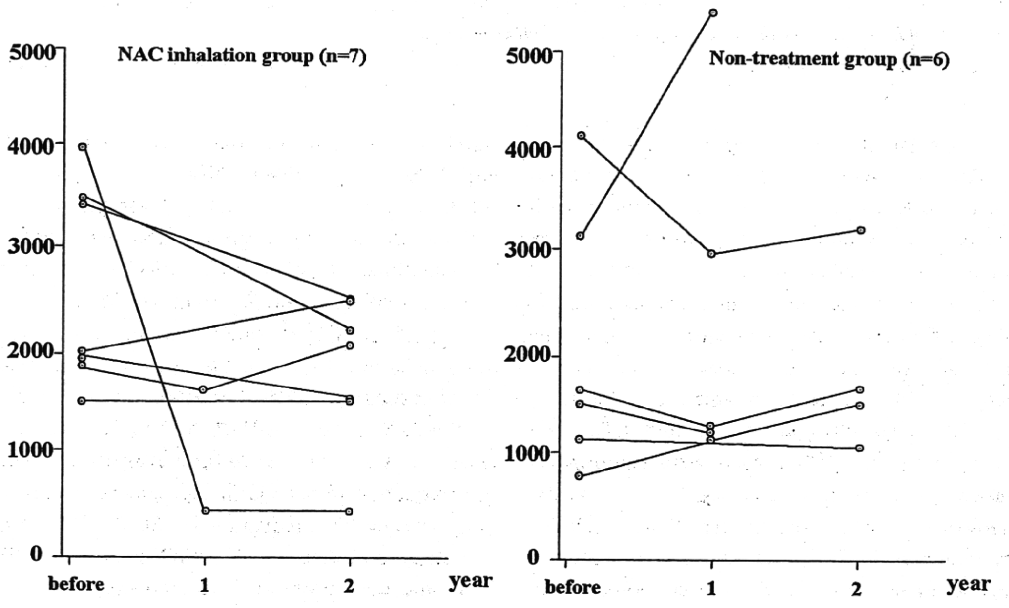

Figure 3. Changes in serum marker KL-6. In one case receiving NAC inhalation, KL-6 improved remarkably after the treatment started, but changes were inconsistent in other cases and there were no significant differences in year-to-year changes between the two groups.

Comparison of Clinical Background between Cases who Continued NAC Treatment and those who did not

Cases that stopped NAC inhalation due to adverse events were compared with those that continued NAC inhalation for more than one year. Due to adverse events, NAC inhalation was stopped in 6 cases in total: 2 in 14 cases that continued NAC for more than one year and 4 in 9 cases that continued NAC inhalation for less than 1 year.

Five of 6 cases who discontinued NAC treatment were females and the 3 patients who developed coughing were female (Table 4). There were no significant differences in clinical background between the two groups except for FVC.

Discussion

In this study, the long-term clinical effects of NAC inhalation monotherapy for IPF were examined in IPF cases who were able to continue the therapy for one year or longer in our department. There were no significant differences in a survival curves between the 14 patients in the NAC inhalation group and the 11 patients in the non-treated group. In addition, significant improvement was not demonstrated by long-term changes in pulmonary function parame-

Table 4. Clinical Background between Cases who Continued NAC Treatment and Those who Did Not

	NAC-continued (n=12)	NAC-discontinued (n=6)
Age (yrs)	62.8 (50-74)	63.2 (45-72)
Sex (n)		
Male	9	1
Female	2	5*
Smoking status		
Current	4	1
Former	4	1
Never	4	4
KL-6 (U/mL)	2606±1282	1632±598
SP-D (ng/mL)	389±73	420±109
LDH (IU/L)	476±142	429±54
FVC (L)	2.62±0.55	1.93±0.74**
%FVC (%)	85.3±12.3	76.0±19.1
%DLco (%)	39.3±16.2	41.8±16.9
PaO_2 (Torr)	83.6±12.5	86.3±4.8

NAC = N-acetylcysteine; FVC = forced vital capacity; %FVC = percentage of predicted forced vital capacity; %DLco = percentage of predicted carbon monoxide diffusing capacity

* $p<0.02$ ** $p<0.05$

ters in the NAC inhalation group compared with the non-treated group. NAC is a precursor of glutathione that attenuates tissue injury due to oxidative stress and exerts an anti-inflammatory effect by inhibiting the expression of inflammatory cytokines and adhesion molecules via inhibition of transcription factor NF-κB-mediated signal transduction. To date, the clinical efficacy of NAC inhalation has only been reported from Japan. Tomioka et al reported a pilot study of aerosolized NAC for IPF of over 12 months (10). This study demonstrated that neither changes in VC and DLco nor quality of life (QOL) in the NAC group were significantly different compared with controls receiving bromhexine hydrochloride. However, it was reported that NAC inhalation suppressed the reduction in SpO_2 after six minutes of walking and improved serum KL-6 levels. Furthermore, in our cases KL-6 levels decreased in 4 cases that included 1 case with a marked decrease. In one case with a marked decrease in KL-6, HRCT showed that ground glass-like appearance was ameliorated. A decrease of circulating KL-6 in IPF is thought to be due to a decrease of KL-6 production by regenerating alveolar type II pneumocyte, and/or to reduced permeability following the destruction of the air-blood barrier in the affected lung (11). Therefore, the treatment has the potential to protect repeated epithelial injury with oxidant-antioxidant imbalance and to delay the progression of IPF.

There were no significant differences in the deterioration of Δ%FVC and Δ%DLco over time between the cases with and without NAC inhalation in the present study. However, this is an open trial and there are limits to the interpretation of the results. First, all of the IPF cases were diagnosed by video-assisted thoracoscopic surgery (VATS) and relatively mild cases accounted for a majority of the cases in this study. According to previous randomized control studies using IFN-γ, pirfenidone, NAC, etanercept and bosentan (3-5, 12, 13), the yearly ΔFVC was presumed to be 160-200 mL in the placebo group. However, since FVC before the beginning of observation varied among cases, Δ%FVC was also variable, ranging from -4.98% to -7.7%. A phase III trial (CAPACITY) carried out in the U.S. revealed that differences in Δ%FVC in the placebo group resulted in different results in %FVC reduction by pirfenidone between CAPACITY 1 and CAPACITY 2 (14). Taken together, it is clear that it is important to fully evaluate the appropriateness of a reduction in %FVC in the placebo group in evaluating drug efficacy by a reduction in %FVC. When the results in this study and those in the IFIGENIA study (3) using oral NAC and corticosteroid were compared, the average yearly reduction in %FVC in the placebo group was -190 mL in the IFIGENIA study, while it was -133 mL and -166 mL in the NAC treatment group and no treatment group, respectively, in the present study, suggesting that NAC inhalation therapy potentially suppresses the decline in FVC over time. NAC inhalation monotherapy may be an effective treatment for mild IPF. However, NAC monotherapy is weakly recommended due to the low quality of the evidence. Therefore, further clinical studies on NAC inhalaton therapy are warranted.

Problems with NAC inhalation include adverse effects, inhalation adherence, and methods and doses of inhalation. In the present study, a number of cases had difficulty in inhaling due to adverse effects at less than one year of inhalation, which should be seriously taken into account. Five of 6 of these patients were females and the 3 patients who developed coughing were female. In terms of inhalation doses, oral NAC at 1,800 mg/day was required to sufficiently ele-

vate glutathione concentrations in the alveolar fluid (15). A small amount of inhalation is expected to exert a beneficial effect, but larger amounts of the drug may reach alveoli under excellent ventilation conditions. Therefore, the optimal dose needs to be defined in future studies.

In this study, the clinical course and changes in the respiratory test results showed various patterns. Martinez et al (16) reported that there were mild reductions in %FVC and %DLco of -3.5% and -0.8%, respectively, in 168 mild to moderate IPF cases followed up for 72 months, but 21% died during the follow-up period and most died from IPF-related causes including acute exacerbation of IPF. Therefore, it is very important to clarify treatment indication in evaluating the efficacy of drug therapy for IPF. As shown in this study, IPF is a disease with markedly heterogeneous manifestations. Mild IPF cases may include cases soon after the onset (early stage cases) and stable cases without progression over years (progression-free cases). In early stage cases, pulmonary function may deteriorate and it is worth investigating the necessity of early intervention such as NAC inhalation. However, in progression-free cases, early intervention may be unnecessary. Taken together, in investigating the efficacy of treatment for mild IPF cases, it is important to take into account the clinical information from six months and one year before treatment, instead of only evaluating clinical information at one time point just before the beginning of treatment, and to evaluate changes over a longer time period in each case. On the other hand, periodical lung function tests were not done after the introduction of NAC inhalation in some cases in this study. As for the potential reasons, some overlapping cases were included in the subjects and there were four cases undergoing long-term oxygen therapy due to advancement of the disease during the follow-up. It is difficult to carry out lung function tests periodically in these cases. In the future clinical study on severe or advanced IPF cases, it is expected that drop-out cases will appear at a certain frequency when lung function tests are adopted for evaluation criteria, which will potentially influence the interpretation of the results.

In this study, among the 14 patients who continued NAC inhalation monotherapy for 1 year or longer, 4 patients (28.6%) developed acute exacerbation of IPF. Since it has been reported that the natural course or progress of IPF varies from one patient to another, the concept of acute exacerbation of IPF is being accepted in the US and Europe (9, 17) The previously reported incidence of acute exacerbation of IPF varied between 5 and 61% depending on the study design and the number of study subjects (17). Risk factors and methods for prevention of developing acute exacerbation are still largely unknown. We also failed to show any significant differences in clinical background or laboratory values before the inhalation treatment between the groups of patients with and without the acute exacerbation of IPF in the present study. In three patients whose pulmonary function parameters were able to be sequentially measured, however, function worsened before developing acute exacerbation. Therefore, attention should be paid to the possible development of acute exacerbation in patients whose pulmonary function worsens even during NAC inhalation therapy.

Lastly, since this study is an open case-control study in a single institute and the number of cases is not large, its use in evaluating the clinical efficacy of NAC inhalation monotherapy is limited. In addition, the role of NAC inhalation in combination with a corticosteroid, an immunosuppressive agent, and a new anti-fibrosis drug should also be investigated, because some mild cases were stable even without treatment, while other cases worsened soon despite NAC inhalation monotherapy.

Acknowledgement

This study was supported by the Health and Labor Sciences Research Grants on Diffuse Lung Diseases from the Japanese Ministry of Health, Labor and Welfare.

Abbreviations: ARDS: acute respiratory distress syndrome, DLco: diffusing capacity for carbon monoxide, ECLIA: electrochemiluminescence immunoassay, ELISA: enzyme-linked immunosorbent assay, FVC: forced vital capacity, IPF: idiopathic pulmonary fibrosis, NAC: N-acetylcysteine, PaO_2: partial pressure of arterial oxygen, QOL: quality of life, SP-D: surfactant protein-D, UIP: usual interstitial pneumonia, VATS: video-assisted thoracoscopic surgery, VC: vital capacity, %DLco: percentage of predicted carbon monoxide diffusing capacity, %FVC: percentage of predicted FVC, %TLC: percentage of predicted total lung capacity

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経気管支肺生検で診断したPulmonary Epithelioid Hemangioendotheliomaの1例

三輪千尋[1]；渡辺恭孝[1]；白石　守[1]；
工藤史明[1]；遠藤俊輔[2]；小山信一郎[1]

自治医科大学附属さいたま医療センター [1]呼吸器科，[2]呼吸器外科

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CASE REPORT

経気管支肺生検で診断したPulmonary Epithelioid Hemangioendotheliomaの1例

三輪千尋[1]；渡辺恭孝[1]；白石　守[1]；
工藤史明[1]；遠藤俊輔[2]；小山信一郎[1]

要約——背景. Pulmonary epithelioid hemangioendothelioma（PEH）は，かつてintravascular bronchioloalveolar tumor（IVBAT）と呼ばれていた疾患で，血管内皮細胞由来の稀な疾患である．**症例.** 29歳女性．5カ月前に結核の曝露歴があり他院で抗結核薬の予防内服をしていた．その後胸部X線，CTにおいて，両側びまん性に小結節影を認め，粟粒結核が疑われ抗結核薬4剤の投与を受けた．しかし，陰影の改善を認めず，喀痰検査でも結核菌は検出されず，転移性肺腫瘍なども疑われ確定診断のために経気管支肺生検（TBLB）を行った．その結果，PEHと診断された．**結論.** 若年女性で胸部X線上，多発小結節影を認め，TBLBにてPEHと診断した．PEHは，TBLBで診断されることは稀であるが，免疫染色などを併用すれば，TBLBによる診断は低侵襲的であり有用であると考えられた．

（気管支学．2010;32:72-77）

索引用語——肺の類上皮血管内皮腫，経気管支肺生検

緒　言

Pulmonary epithelioid hemangioendothelioma（PEH）は，かつてintravascular bronchioloalveolar tumor（IVBAT）と呼ばれていた疾患で，血管内皮細胞由来の稀な疾患である．今回我々は胸部X線上，両側肺野の多発小結節影を認め，経気管支肺生検（TBLB）により診断し得たPEHの1例を経験したので報告する．

症　例

症例：29歳女性．

主訴：胸部異常影（自覚症状なし）．

現病歴：5カ月前に夫が肺結核と診断されたため，他院で抗結核薬の予防内服をしていた．その後，胸部X線写真にて，両側多発性の小結節影を指摘され，粟粒結核を疑われ，抗結核薬4剤の内服を開始した．しかし，胸部X線上改善を認めず精査目的で当院に紹介された．

既往歴：流産．

内服薬：RFP（rifampicin），INH（isoniazid），EB（ethambutol），PZA（pyrazinamide）．

家族歴：父 高血圧，夫 結核．

生活歴：喫煙なし．

来院時身体所見：身長155 cm，体重53 kg，BMI 22.1 kg/m^2，SpO_2 99%（室内気吸入下），体温36.2℃，血圧126/78 mmHg，脈拍80回/分，整，結膜に貧血，黄疸なし，頸部リンパ節触知せず，胸部聴診ラ音なし，心雑音なし，腹部所見なく下腿浮腫なし，意識清明で神経学的所見異常なし．

来院時検査所見（Table 1）：末梢血液像，血沈，血液生化学，腫瘍マーカー，肺機能検査はすべて正常範囲内で，喀痰培養では異常を認めなかった．

来院時胸部X線写真（Figure 1）：両側にびまん性に，10 mm以下で大小不同の小結節影を認めた．

来院時胸部CT写真（Figure 2）：全肺野で両側多発性に，気管支や血管走行，小葉構造とは一定の関連を持たない大小不同で境界明瞭な小結節を認め，画像上，転移性肺腫瘍や悪性リンパ腫などの腫瘍疾患や粟粒結核やサルコイドーシスなどの肉芽腫性疾患などを疑い，確定診断のためB^{2}b，B^{3}a，B^{4}a，B^{6}b，B^{8}aから無作為にTBLBを施行した．ヘマトキシリン-エオジン染色弱拡大では，細胞密度は中等度で，硝子化傾向のある結節性病変を認め，辺縁では肺胞腔内進展が認められた（Figure 3）．強拡大では，硝子化した基質を背景にクロマチンが増加し，核縁が不整な腫瘍細胞を認め（Figure 4A），免疫染色では内皮細胞マーカーであるCD34（Figure 4B）やCD31に陽性であり，PEHと組織診断された．他の臓器への転

自治医科大学附属さいたま医療センター[1]呼吸器科，[2]呼吸器外科．
受付日：2009年3月13日，採択日：2009年9月1日．

Table 1. Laboratory Findings on First Visit

Hematology		BUN	12 mg/dl
WBC	9150/μl	Cr	0.66 mg/dl
Neut	87.0%	Na	140 mmol/*l*
Lymp	7.0%	K	3.8 mmol/*l*
Mono	5.0%	Cl	104 mmol/*l*
Baso	1.0%		
RBC	490/μl	Tumor markers	
Hb	14.0 g/dl	CEA	1.5 ng/ml
Ht	42.7%	CA19-9	≧ 2.0 U/ml
Plt	25.7×10^4/μl	CA125	10.9 U/ml
ESR	16 mm/h	SCC	≧ 0.5 ng/ml
Biochemistry		Sputum culture	
TP	7.6 g/dl	Acid fast bacteria stain	negative
Alb	4.9 g/dl	Pulmonary function	
T-bil	0.66 mg/dl	%VC	110.3%
AST	21 mU/ml	$FEV_{1.0\%}$	89.3%
ALT	20 mU/ml	$FEV_{1.0}$	2.85 *l*
LDH	146 mU/ml	Tuberculin reaction	positive
ALP	299 mU/ml		
γ-GTP	17 mU/ml		

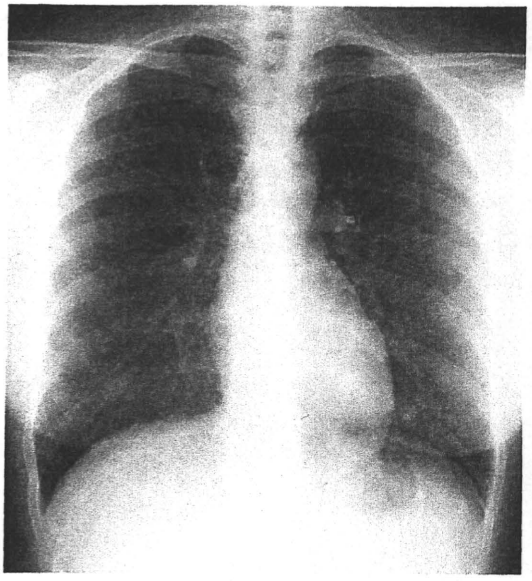

Figure 1. Chest radiograph on first visit showed multiple bilateral pulmonary nodules.

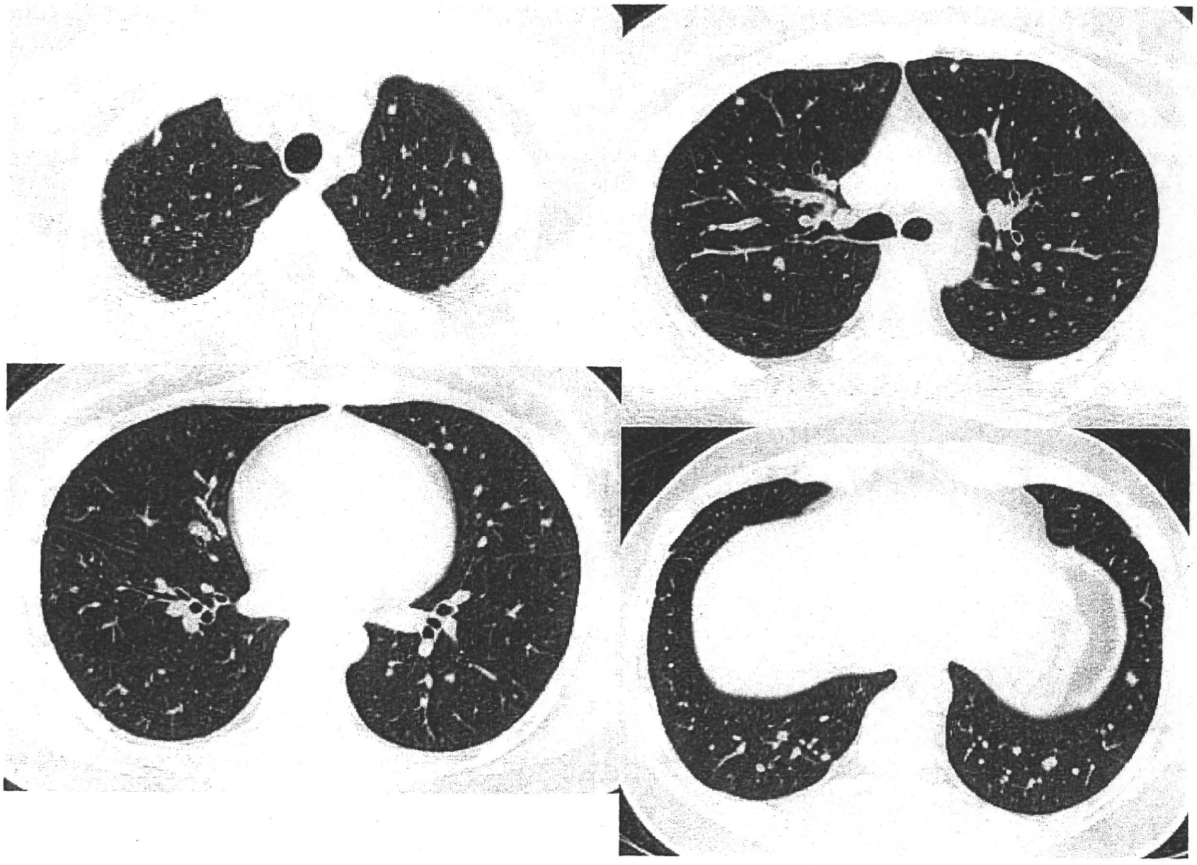

Figure 2. Chest CT scan demonstrated multiple bilateral small pulmonary nodules (under 10 mm) not associated with bronchi or veins.

移を調べるために行った腹部のダイナミック造影CTでは，肝臓S4に2 cm大の，辺縁から徐々に造影される造影効果を伴う腫瘤性病変を認めた．肝生検は行っておらず，確定診断はついていないが，画像上はPEHの転移として矛盾しない所見であった（Figure 5）．治療に関しては，本症例では，肝臓に転移を疑う所見は認めるものの，肺については無症状でびまん性多発結節のみであることから経過観察としているが，半年経過した現在腫瘍の増大は認めていない．

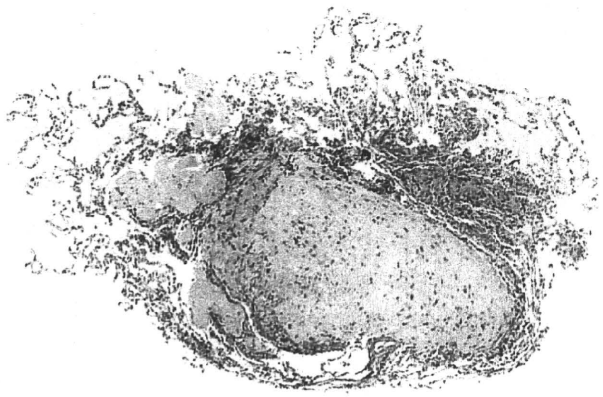

Figure 3. Histology with hematoxylin and eosin staining. Low magnification view of the transbronchial lung biopsy specimen shows a nodular lesion composed of hyalinous matrix and moderate cellularity.

考　察

Pulmonary epithelioid hemangioendothelioma（PEH）は，組織学的特徴からDailとLiebowらによってIVBATとして初めて報告された疾患である[1,2]．彼らは腫瘍細胞が肺胞上皮由来のものであると考えていたが，Corrinら[3]やWeldon-Linneら[4]は電子顕微鏡観察を行い，腫瘍細胞の細胞質内にWeibel-Palade bodyを見出し，血管内皮細胞由来とした．その後，血管内皮細胞内で合成される第VIII因子が免疫組織化学法により腫瘍細胞内に証明されたことより，血管内皮細胞由来の腫瘍とされた[5]．

Aminら[6]の報告によると，PEHの平均年齢は40.1±17.5歳で73%は女性である．半数は無症状であるが，咳嗽18.3%や胸痛16%のような非特異的な症状がある．画像的にはPEHは結節影を認め，84%の症例で多発し，大きさ（0.5～15 cm）は様々であるが，多くは2 cm以下の小結節であった．この結節は両側性が78.9%，片側性12.2%，単発8.9%である．軟部組織以外の好発部位としては肺，肝，骨などが挙げられその他に口蓋扁桃[7]，脳[8]，前立腺[9]発生の報告がある．

現在までの本邦におけるPEHの報告例は，自験例を

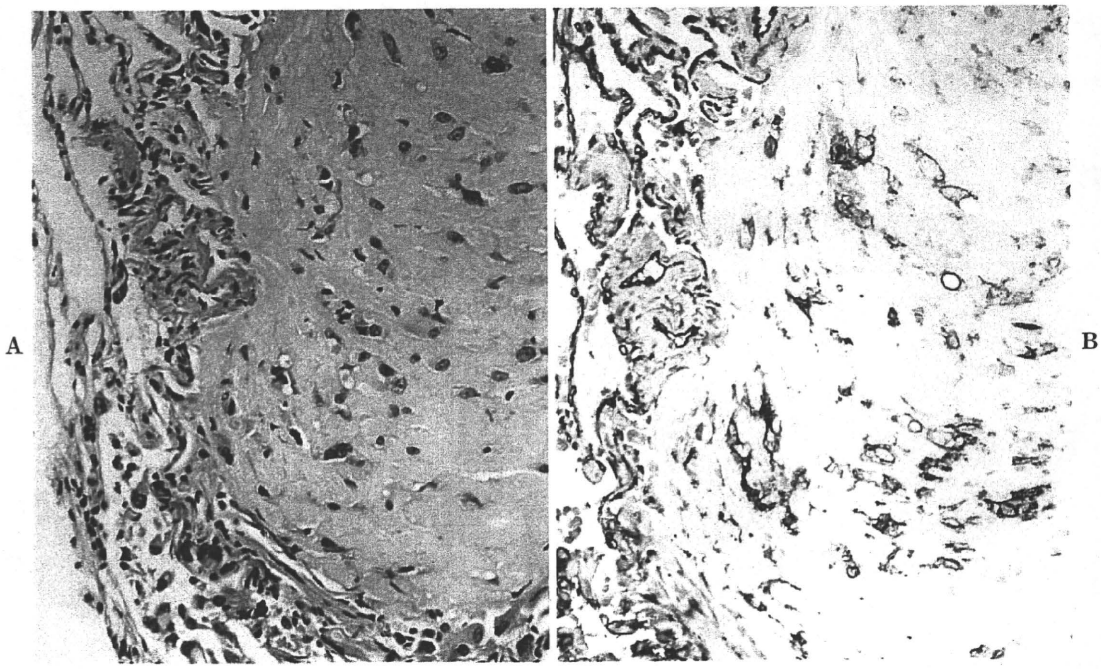

Figure 4. **A:** Histology with hematoxylin and eosin staining. High magnification view of the lesion shows tumor cells with increased chromatin particles and irregular nuclei embedded in hyaline matrix. **B:** Histology with CD34 immunostaining. Tumor cells (right) and endothelia of alveolar septa (left) stain positively for CD34.

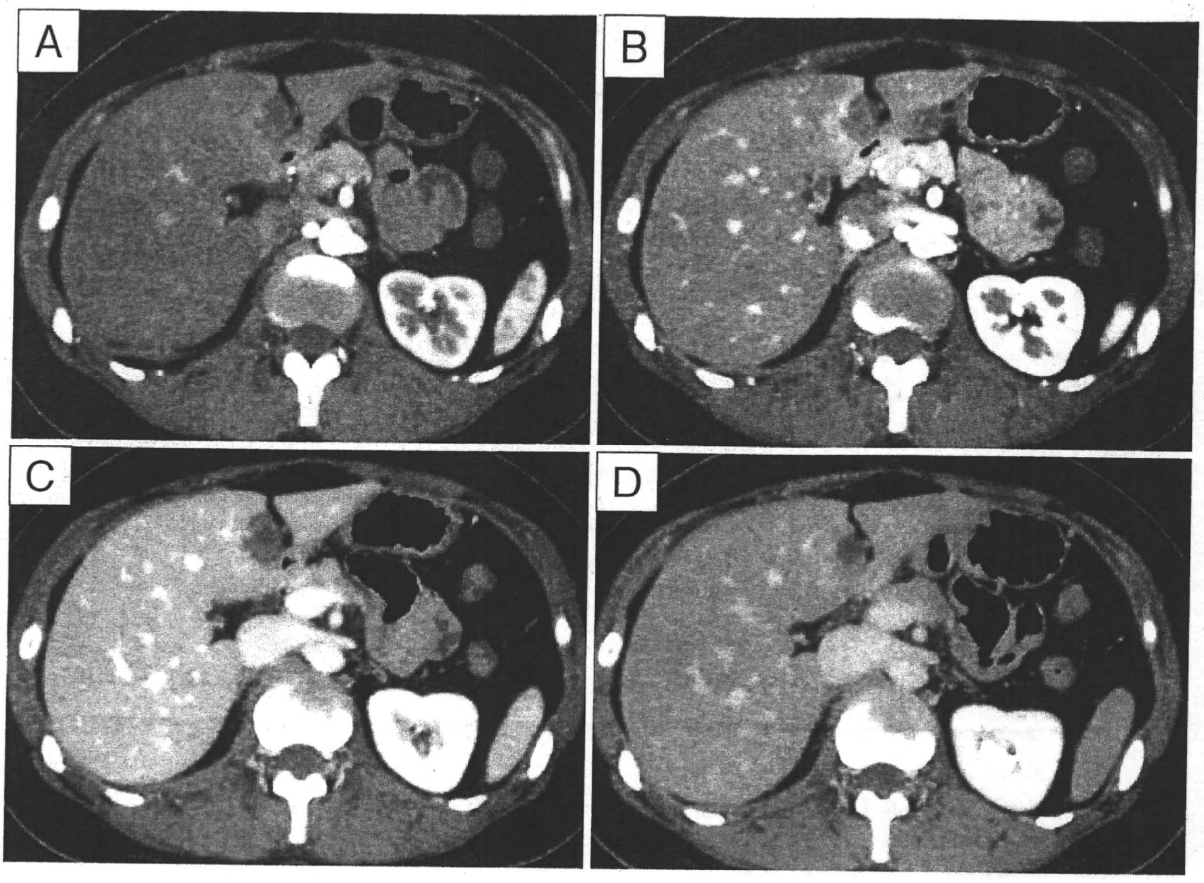

Figure 5. Abdominal contrast CT. Scans were obitained during injection of 100 ml of contrast medium at 3.0 ml/sec. After the start of the injection, precontrast CT was initiated at 13 seconds **(A)**, hepatic arterial CT at 24 seconds **(B)**, and portal venous CT at 65 seconds **(C)**, venous CT at 180 seconds **(D)**. This study shows low density area with gradual enhancement from the margin. The lesion was suspected to be metastasis from pulmonary epithelioid hemangioendothelioma (PEH) in the liver.

含めて53例で，海外における報告例も調べた限りでは91例と稀な疾患である．本邦における報告のまとめをTable 2に示した．平均年齢は44.2±16.9歳で，性差は男性45.3%(24例)，女性54.7%(29例)と女性の方が多かったが，女性が80%を占めるDailら[2]の報告に比べ低い値となっている．また発見時の症状については，58.5%が無症状であったが，海外の報告例では，無症状は33.7%のみで自覚症状がある場合が多く，咳嗽，胸痛，血痰，呼吸困難などが見られた．胸部X線写真上の特徴としては多発結節陰影が41例（77.3%）と多く，56.4%で10 mm以下の結節影であった．診断は，外科的肺生検（開胸肺生検，胸腔鏡下肺生検）で確定されることが多い．気管支鏡検査を施行したのは27例で，そのうち内腔観察のみ行った例が3例，TBLB 18例，擦過細胞診4例，洗浄細胞診1例，詳細不明は2例であった(Table 3)．擦過細胞診や洗浄細胞診では，確定診断に至らなかった．TBLB施行例では18例中14例で確定診断がつかず，2例は肺癌や中皮腫などの悪性腫瘍と診断された．自験例を含め2例のみがTBLBによってPEHと診断された[10]（Table 3)．肝生検や皮膚生検など肺以外の部位からも組織診が行われたのは11例で，そのうち4例はPEHと診断がついた．

本症の確定診断はTBLBでは難しく，外科的肺生検でなされることが多い．現在までの海外の報告例でもTBLBで診断できた例は2例と少ない．その理由としてPEHは胸部X線上，小結節影を呈することが多くTBLBでは，X線透視下で病変部位を確認し生検することが難しいこと，その上，無作為な生検ではPEHのような腫瘍性病変は広義間質に進展するサルコイドーシスなどの肉芽腫性病変と異なり，病変が捉えにくいことが考えられる．しかし，TBLBで悪性腫瘍と診断された症例では，何らかの病変を採取できており，PEHを疑い適切な免疫染色を行うことで，診断がつく可能性があると思われた．細胞診では，腫瘍細胞，特に腺癌との鑑別が困

Table 2. Data: 53 Cases of Pulmonary Epithelioid Hemangioendothelioma (PEH) from 1983 to 2008 in Japan, Including Our Case

Sex Male:Female	24:29	Extra pulmonary lesions		Therapy	
Age (Yrs)		Liver	10	Follow-up	27
All mean ± SD	44.2 ± 16.9	Skin	4	Operation	10
Male mean ± SD	44.3 ± 17.7	Bone	3	Chemotherapy	5
Female mean ± SD	44.2 ± 16.5	Chest wall	3	Radiotherapy	3
Initial symtoms		Others	3	Others	4
No symptoms	31	Intraperitoneal	2	Not described	9
Cough	7	Muscle	1	Prognosis	
Chest pain	6	Palate	1	Alive	31
Bloody sputum	4	Brain	1	Dead	14
Back pain	3	Not described	9	Unknown	8
Dyspnea	1	Method of diagnosis		Cause of death	
Others	6	Open lung biopsy	19	Respiratory failure	8
Radiographic findings		VATS	14	Intraperitoneal metastasis	2
Nodular numbers		Lung resection	13	Wide spread of tumor	2
Multiple	41	Transbronchial lung biopsy	2	Meningitis	1
Single	11	Autopsy	3	Cardiac infarction	1
Tumor size > 10 mm	17	Others (liver 2, skin 1, soft tissue 1)	4		
≤ 10 mm	22				
Not described	13	Not described	1		
Pleural effusion	7				

Table 3. Cases in Table 2 That Underwent Bronchoscopic Examination

Bronchoscopic examination	27
Transbronchial lung biopsy	18
No diagnosis	14
Epithelioid hemangioendothelioma	2
Adenocarcinoma	1
Other malignancy	1
Sputum cytology	4
Bronchoalveolar lavage	1
Only followed	3
Not described	2

難であるが，PEH の特徴として胞体内空胞があり，腺癌細胞と比較して細胞の形状は類円形から紡錘形で，胞体は広く，薄く，辺縁は不規則で不明瞭であり，核小体は比較的小型である[11,12]．また，診断には，factor VIII-related antigen, CD34 などの血管内皮マーカー染色が有用な可能性があると報告されている[5,9]．また，気管支鏡検査で診断がつかなかった症例のうち，後に外科的肺生検を行った症例において 2 例[13,14]で組織診断と比較検討されており，空胞を持つ腫瘍細胞を認め PEH の診断との一致が確認されている．本症例では，擦過細胞診も行っているが，異型細胞を認めなかった．

治療は半数で経過観察のみで，抗癌剤，放射線治療などの施行例もある．なお，PEH に対し bevacizumab の投与で進行を抑制できている報告[15]もあり，PEH は血管内皮細胞由来の腫瘍であることから今後新たな治療法として期待される．本症例も今後，治験への参加を考慮中である．本邦での予後は，53 例中 14 例死亡しており，また発見から死亡までは 2 カ月から 12 年であり，死因としては呼吸不全が多かった．

若年女性で胸部 X 線上，多発小結節影を認めた場合，PEH も疑って，まずは低侵襲的な TBLB を行い，必要な免疫染色を行うことで診断されうる可能性があることが示された．

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A Case of Pulmonary Epithelioid Hemangioendothelioma Diagnosed by Transbronchial Lung Biopsy

Chihiro Miwa[1]; Yasutaka Watanabe[1]; Mamoru Shiraishi[1]; Fumiaki Kudo[1]; Syunsuke Endo[2]; Shinichiro Koyama[1]

ABSTRACT —— ***Background.*** Pulmonary epithelioid hemangioendothelioma (PEH), previously termed intravascular bronchioloalveolar tumor, is a rare malignancy now known to originate from hemangioendothelial cells. ***Case.*** A 29-year-old woman had been under antituberculosis drugs treatment due to prior exposure to tuberculosis 5 months before. Chest X-ray and chest CT showed diffuse bilateral multiple small nodules, and miliary tuberclosis was suspected. She was started on 4 types of antituberculosis drug. However her chest X-ray did not improved. Furthermore, tubercule bacillus was not found in her sputum. Therefore we suspected metastatic cancer and performed a transbronchial lung biopsy (TBLB), which led to a diagnosis of PEH. ***Conclusion.*** In a young woman whose chest X-ray showed multiple small nodular shadows, TBLB was used to diagnose PEH. TBLB is a useful in conjunction with immunostaining, low invasive examination in the diagnosis of this rare case of PEH.

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KEY WORDS —— Pulmonary epithelioid hemangioendothelioma, Transbronchial lung biopsy

[1]Department of Pulmonary Medicine, [2]Department of Thoracic Surgery, Jichi Medical University Saitama Medical Center, Japan.
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