contralateral hilar lymph nodes; no prior treatment; life expectancy of at least 2 months; the Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 2; at least one bidimensionally measurable lesion; age less than 80; adequate organ function, such as white blood cell (WBC) count of 4000×10^6/L or greater, hemoglobin level 10 g/dL or greater, platelet count 100×10^9/L or greater, AST and ALT less than 100 IU/L, bilirubin level 1.5 mg/dL or less, creatinine concentration 1.2 mg/dL or less, electrocardiogram (ECG) findings within normal range, and left ventricular ejection fraction (LVEF) of echocardiogram 60% or greater. All patients gave written informed consent. Ineligibility criteria were: brain or bone metastases requiring radiation; continuous long-term treatment with non-steroidal anti-inflammatory drugs and glucocorticoids; pulmonary fibrosis; serious complications and other active malignancy; or pregnant or nursing subjects.

This study was approved by the institutional review boards at each participating center.

Study design

Amrubicin (Sumitomo Pharmaceuticals Co., Ltd, Osaka, Japan) was dissolved in 20 mL normal saline and administered once intravenously as a 5-min infusion at a dose of 45 mg/m^2/day on days 1 to 3, every 3 weeks.

Before treatment, all patients underwent a medical history, physical examination, hematology and serum biochemistry tests, urinalysis, ECG, LVEF, and baseline tumor measurements (chest radiography, CT scans, bone scintigraphy, and other measurements as appropriate). All measurable and assessable lesions were evaluated within 2 weeks before treatment. ECG and LVEF were undertaken within 1 month before treatment.

Complete and differential blood cell counts, platelet counts, hematocrit analysis, biochemical analysis including AST, ALT, alkaline phosphatase, LDH, total bilirubin, BUN, creatinine, serum bilirubin, albumin, total protein, and electrolyte levels (Na, K, Cl, and Ca), and urinalysis (including protein, glucose, urobilinogen, and occult blood) were performed weekly as a rule. When severe myelosuppression was observed, complete and differential blood cell counts plus platelet counts were performed 2 times or more per week. ECG was undertaken every treatment cycle and LVEF every other cycle. Chest radiography and CT scans were carried out every cycle as a rule.

Subjective and objective symptoms were observed and recorded as appropriate.

Dose modifications were made according to WBC and platelet counts. If the WBC count nadir was lower than $1{,}000 \times 10^6$/L for 4 days or longer and/or the platelet count nadir was lower than 50×10^9/L, a dose reduction of 5 mg was stipulated in the subsequent treatment course. Treatment was postponed until the WBC and platelet counts recovered to $\geq 3{,}000 \times 10^6$/L and $\geq 100 \times 10^9$/L, respectively.

In patients who demonstrated tumor regression of 25% or greater after the first course of chemotherapy, amrubicin treatment was continued. After the second course, patients had to have achieved tumor regression of 50% or greater to continue to receive the drug up to a maximum of 6 courses. Treatment of combination chemotherapy with etoposide (100 mg/m^2 on days 1, 2, and 3) and cisplatin (80 mg/m^2 on day 1) was recommended for patients who failed to fulfill any of the above criteria.

Evaluation of response and toxicity

Response was assessed according to the "Criteria for the evaluation of the clinical effects of solid cancer chemotherapy" of the Japan Society for Cancer Therapy [14], which are virtually identical to those of the World Health Organization [15]. A complete response (CR) was defined as disappearance of all lesions for a minimum of 4 weeks. A partial response (PR) was defined as a 50% or greater decrease in the sum of the products of the diameters of measurable lesions for a minimum period of 4 weeks and no new lesions. No change (NC) was defined as a decrease in the tumor mass of less than 25% or any increase of less than 25%. Progressive disease (PD) was defined as an increase in the size of any measurable lesion by 25% or greater or the appearance of new lesions.

Toxicity grading was recorded based on the side effect record form in the "Criteria for the evaluation of the clinical effects of solid cancer chemotherapy" of the Japan Society for Cancer Therapy [14].

Statistical analyses

The estimated sample size was 30 to guarantee that the lower limits of 95% confidence interval would be at least 20% at 40% of expected response rate. An early cessation rule was in place to terminate the study if at least 4 responses had not been seen among 15 patients evaluated. Median overall survival was estimated using the product-limit (Kaplan-Meier) method [16].

Results

Patient characteristics

Of 35 patients entered into this study between May 1995 and January 1997, 33 patients were eligible and assessable for efficacy and toxicity. There were 2 ineligible patients because of serious complications before treatment (cardiac

Table 1 Patient characteristics

Patient characteristics	No. of patients (N = 33)	%
Age (years)		
Median	66	
Range	42–78	
Sex		
Male	29	87.9
Female	4	12.1
Performance status (ECOG)		
0	5	15.2
1	26	78.8
2	2	6.1
Stage		
IIIB	1	3.0
IV	32	97.0
Prior therapy		
No	33	100

ECOG: Eastern Cooperative Oncology Group.

failure and aggravation of hepatitis, respectively), and they did not receive amrubicin. Characteristics of the 33 eligible patients are shown in Table 1. Of the 33 patients, 13 (39%) were 70 years of age or older, 88% were male, and 94% had an ECOG performance status of 0 or 1.

Efficacy

Response to amrubicin is shown in Table 2. The early cessation rule was not imposed to terminate the study, as 10 responses were seen after 15 patients were enrolled. Of 33 patients, 3 achieved a complete response, giving a CR rate of 9.1% (95% CI, 1.9–24.3%), and 22 a partial response, for an overall response rate of 75.8% (95% CI, 57.7–88.9%). Of 7 patients, 6 experiencing no change under amrubicin treatment were switched to salvage chemotherapy. Of these, 2 had partial responses and the others had no change.

The overall survival curve is shown in Fig. 2. Median survival time was 11.7 months (95% CI, 9.9–15.3 months), and 1-year and 2-year survival rates were 48.5% (95% CI, 31.4–65.5%) and 20.2% (95% CI, 6.4–34.4%), respectively.

Toxicity

The major observed toxicity was hematologic, as shown in Table 3. All patients experienced leukopenia and neutropenia. Grade 3 or 4 leukopenia occurred in 51.5% of patients and grade 3 or 4 neutropenia in 84.8%. Anemia and thrombocytopenia were observed in 78.8% and 39.4% of patients, respectively, both with a frequency of grade 3 or 4 of 21.2%. Despite the severe hematologic toxicity of amrubicin, there was no febrile neutropenia or treatment-related death during the entire treatment of 33 patients. Granulocyte colony-stimulating factor (G-CSF) was used in 55 (40%) of a total of 136 cycles, in 13 patients (39%). Most hematologic toxicity in this trial was well-controlled without dose reduction: 88% of the total treatment cycles were delivered at the planned dosage of amrubicin, 45 mg/m^2/day.

Non-hematologic toxicities observed in more than 10% of patients were anorexia (54.5%), nausea and vomiting

Table 2 Response to amrubicin

No. of assessable patients	Response (No. of patients)				CR rate, % (95% CI)	Response rate, % (95% CI)
	CR	PR	NC	PD		
33	3	22	7	1	9.1 (1.9–24.3)	75.8 (57.7–88.9)

CR: complete response; PR: partial response; NC: no change; PD: progressive disease; 95% CI: 95% confidence interval.

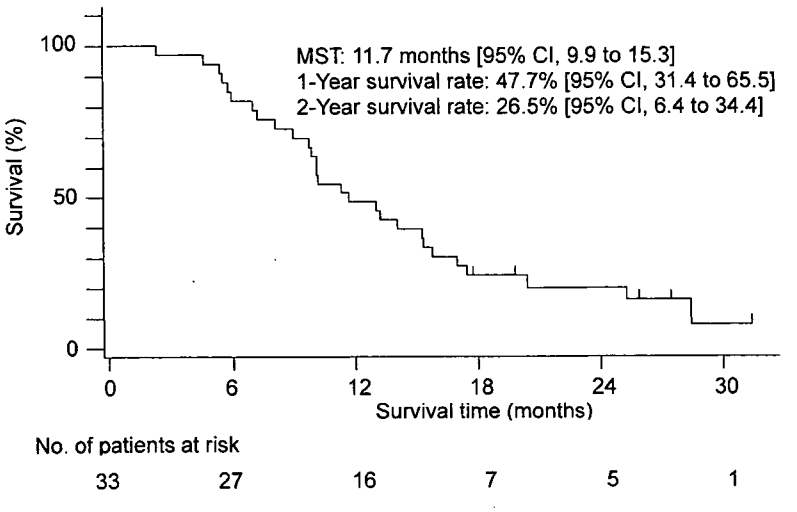

Fig. 2 Overall survival of patients with extensive-disease small cell lung cancer treated with amrubicin. MST: median survival time; 95% CI: 95% confidence interval

Table 3 Main treatment-related toxicity of amrubicin

Toxicity	No. of assessable patients	Toxicity grade others					
		1	2	3	4	≥ 1	≥ 3
		(No. of patients)				Frequency (%)	
Hematologic toxicity							
Anemia (hemoglobin)	33	12	7	6	1	78.8	21.2
Leukopenia	33	5	11	13	4	100	51.5
Neutropenia	33	1	4	14	14	100	84.8
Thrombocytopenia	33	3	3	1	6	39.4	21.2
Non-hematologic toxicity							
Stomatitis	33	2	1	0	0	9.1	0
Anorexia	33	12	3	3	–[a]	54.5	9.1
Nausea and vomiting	33	12	7	0	–[a]	57.6	0
Diarrhea	33	6	0	0	0	18.2	0
Fever	33	3	7	0	0	30.3	0
Phlebitis	33	1	1	0	0	6.1	0
Alopecia	33	11	8	1	–[a]	60.6	3.0
Total bilirubin elevation	33	1	1	0	0	6.1	0
AST elevation	33	5	0	0	0	15.2	0
ALT elevation	33	8	1	0	0	27.3	0
ALP elevation	33	1	0	0	0	3.0	0
BUN elevation	33	2	0	0	0	6.1	0
Others[b]	Headache, 1/33[c]; Rash, 1/33; Constipation, 1/33; Interstitial pneumonia, 1/33; Rhinorrhagia, 1/33; ECG abnormality, 3/32						

AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; BUN: blood urine nitrogen; ECG: electrocardiogram.

[a]Toxicity grade was not defined for these toxicities.

[b]Toxicities which were not graded.

[c]Proportion of number of reported patients to number of observed patients.

(57.6%), diarrhea (18.2%), fever (30.3%), alopecia (60.6%), AST increase (15.2%), and ALT increase (27.3%). Most of these were mild (≤grade 2), with only 3 patients (9.1%) experiencing grade 3 anorexia and 1 patient grade 3 alopecia (3.0%). A single patient developed interstitial pneumonia after the second cycle of treatment; however, it was reversibly recovered by steroid therapy and cessation of amrubicin treatment. ECG abnormality was observed in 3 patients (9.4%; supraventricular extrasystole, prolonged QT interval, and T wave flattening in 1 patient each), which did not need any treatment. No LVEF decrease was observed.

Discussion

Results of this phase II study demonstrate that amrubicin is an extremely active agent against extensive-disease SCLC. The complete response rate was 9.1% (95% CI, 1.9–24.3%), overall response rate 75.8% (95% CI, 57.7–88.9%), and median survival time 11.7 months (95% CI, 9.9–15.3 months). These results are comparable or even superior to those of the standard combination regimen of cisplatin and etoposide, used as the gold standard of extensive-disease SCLC therapy since 1981 and remaining unchanged over the last 2 decades [4].

SCLC is sensitive to cytotoxic anticancer agents. Of anticancer drugs developed before 1990, a number of agents with response rates of 20% or greater for SCLC were listed as active drugs [17]. Of these drugs, etoposide, cisplatin, carboplatin, doxorubicin, cyclophosphamide, and vincristine, are still currently used as important constituents of combination regimens in the treatment of SCLC. In addition, several drugs with significant activity for SCLC have been developed since 1990. Irinotecan showed a response rate of 33% to 47% even in previously treated patients who are generally less sensitive to chemotherapy [18, 19]. Recently a new combination regimen of irinotecan plus cisplatin was demonstrated to be significantly superior to standard regimen of etoposide plus cisplatin in median survival time (12.8 months vs. 9.4 months, $P = 0.002$) [3]. In addition, topotecan, paclitaxel, docetaxel, and gemcitabine are reported to have response rates of 26% to 41% for extensive-disease SCLC patients without previous treatment [20–24]. Compared to these agents, amrubicin demonstrated a much higher response rate (75.8%) in this study, indicating it is a promising novel agent with potential to overcome the therapeutic plateau of SCLC.

The major toxicity of amrubicin was hematologic. Grade 3 or 4 leukopenia was frequently observed in 51.5% of patients and grade 3 or 4 neutropenia in 84.8% of patients. Despite such severe hematologic toxicity, 88% of the total treatment cycles could be delivered without dose reduction and non-hematologic toxicities were mild. Although anorexia (54.5%) and nausea and vomiting (57.6%) were frequently observed, there were no episodes of grade 3 or 4 toxicity, except for 3 patients (9.1%) with grade 3 anorexia and 1 patient (3.0%) with grade 3 alopecia. A single patient developed interstitial pneumonia; however, this was reversible with steroid therapy. ECG abnormalities were observed in 3 patients, but they were each reviewed by a medical cardiologist and judged not to be clinically significant. No LVEF decrease was observed. Results show that the toxic profiles of amrubicin are acceptable and favorable in the treatment of extensive-disease SCLC, although due to its hematologic toxicity, in particular neutropenia, G-CSF support is needed.

In conclusion, amrubicin is a very active and promising agent with acceptable toxicity for patients with SCLC. Further studies are warranted in combination with other agents for this disease.

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Clinical Experience with Autofluorescence Imaging System in Patients with Lung Cancers and Precancerous Lesions

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Key Words
Lung cancer · Autofluorescence endoscopy · Autofluorescence imaging system

Abstract
Background: It is important to detect preinvasive bronchial lesions before they become invasive cancer, because detection of early cancer is expected to lead to a cure. Autofluorescence bronchoscopy is a useful device in the detection of preinvasive and cancerous lesions. Recently, a new autofluorescence bronchoscopic system, autofluorescence imaging (AFI) system, has been developed. ***Objectives:*** We evaluated the efficacy of AFI in the diagnosis of precancerous and cancerous lesions. ***Methods:*** A total of 31 patients underwent both conventional white-light bronchoscopy (WLB) and AFI from January 2002 to September 2004. We evaluated autofluorescence findings using a four-point scale: AFI-I, II, III, and B. The findings in WLB were evaluated on a three-point scale: WLB-I, II, and III. Abnormal areas by WLB and AFI were biopsied for histopathological examinations. ***Results:*** A total of 64 lesions were evaluated. When the AFI-III finding was regarded as positive in AFI and WLB-III as positive in WLB, sensitivity for severe dysplasia or worse was 94.7% with AFI and 73.7% with WLB, respectively. ***Conclusions:*** AFI is an effective system for the detection of precancerous and cancerous lesions.

Introduction

Both the incidence and mortality rate of lung cancer are increasing. In 1998, lung cancer deaths first surpassed deaths from gastric cancer, and became the leading cause of death from cancer in both men and women in Japan. Detection of cancer, especially carcinoma in situ (CIS) and microinvasive carcinoma, has been widely expected to lead to a cure. Endobronchial treatments such as photodynamic therapy and brachytherapy have improved the cure rate of the central type of early-stage lung cancer [1–3]. Before endobronchial treatments became clinically applicable, surgical resection was a standard treatment for this stage of lung cancer. Recently, these treatments are considered to be good alternatives for surgery because they can preserve lung volume. Since it is difficult even for experienced bronchoscopists to identify dysplastic and early-stage carcinomatous lesions including CIS by conventional white-light bronchoscopy (WLB) alone, fluorescence endoscopy has been applied to detect these le-

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sions. The laser-induced fluorescence endoscopy (LIFE) system (Xillix, Vancouver, Canada) is one of the autofluorescence bronchoscopic systems developed by Hung and Lam et al. [4–6]. LIFE has been developed to detect the lesions of dysplasia, CIS, and cancers. Recently, a new autofluorescence bronchoscopic system, autofluorescence imaging (AFI), has been developed by Olympus (Olympus Medical Systems Corp., Tokyo Japan). AFI utilizes a videoscope system, and second-generation AFI is capable of displaying both the images of conventional mode and AFI mode on the same monitor by use of a switch. In AFI mode, a combination image of autofluorescence signal and two independent signals from two restricted wavelengths is displayed. By adding autofluorescence information to high-resolution images by conventional mode, holistic diagnosis of endobronchial lesions is expected to become possible. In this paper, we evaluated the efficacy of AFI in the diagnosis of precancerous and cancerous lesions.

Subjects and Methods

AFI System

A charge-coupled device is equipped with filters that cut excitation light in the optical system of the AFI bronchovideoscope to construct images by a 460–690 nm wavelength. The outer diameter of the distal end of the AFI bronchovideoscope is 6.1 mm. From a light source equipped with a 300 W xenon lamp, three lights are irradiated sequentially in AFI mode – the excitation light (395–445 nm) for taking autofluorescence images, and G′-light (550 nm) and R′-light (610 nm) for taking reflected images (G′-image and R′-image, respectively) [7]. Both autofluorescence (460–690 nm) signals and the reflected signals of G′-light and R′-light are processed in the videoprocessor, and composite images are displayed on the monitor as AFI images. The wavelengths of G′-light and R′-light are determined by the hemoglobin's absorption features. Hemoglobin absorbs a large volume of G′-light wavelength and the areas containing more hemoglobin are displayed dark in the G′-image. Consequently, the blood volume (hemoglobin quantity) in the tracheobronchial mucosa is well reflected in the G′-image. On the other hand, hemoglobin absorbs a lesser quantity of R′-light wavelength, and R′-images can afford a basic intensity of signals from the mucosa independently of blood supply [7]. Allocating autofluorescence images to green, G′-images to red, and R′-images to blue, the combined images are constructed by standardizing the intensities of autofluorescence images and G′-images by that of R′-images. AFI displays a green image for normal epithelium. The abnormalities in AFI are basically classified into two patterns, the area expressing magenta color where red is relatively dominant and the area expressing blue color where blue is relatively dominant. In the first generation, AFI lacked the switch that changed two modes of observation images, conventional and AFI mode. In the second generation, a switch was added allowing for the alternating between two modes.

Subjects

A total of 31 patients with risk factors for central-type early lung cancer, 30 men and 1 woman, underwent AFI in our hospital from January 2002 to September 2004. The reasons patients underwent AFI are classified as follows: 10 patients with positive sputum cytology, 8 patients with symptoms including bloody sputum and cough, 11 patients as monitoring after previously treated lung cancer (photodynamic therapy 6, radiotherapy 1, surgical resection 4), and 2 patients as an examination of thoracic malignancy. The mean age was 69 (range 57–80) years old.

Methods

After informed consent was obtained, both WLB and AFI were performed under local anesthesia. We used bronchoscopes (Olympus BF200 or BF240) as WLB and a AFI device as autofluorescence bronchoscopy. Using the first generation of the AFI system, we performed WLB followed by AFI. In this series, 9 patients were enrolled from January to October in 2002. To avoid the bias by bronchoscopic procedure, we performed AFI followed by WLB when the second generation of the AFI device was used. In this series, 22 patients were enrolled from October 2003 to September 2004.

We evaluated the autofluorescence findings using a four-point scale. These evaluations for lesions were done by two or more bronchoscopists. AFI-I images had normal autofluorescence intensity and were green, AFI-III images had much less autofluorescence intensity than AFI-I and appeared magenta. AFI-II images fell in between AFI-I and AFI-III and appeared pinkish-brown. When increased blue color was observed in a lesion, AFI-B was allocated. However, if the lesion showed AFI-II or III in blue image, AFI-II or III was allocated.

Similarly, the findings in WLB were evaluated on a three-point scale. WLB-I was defined as normal mucosa, WLB-II as slightly abnormal mucosa (changes suggesting mild/moderate dysplasia), and WLB-III as severely abnormal mucosa (changes suggesting severe dysplasia and cancer). All abnormal areas with a scale of II or III or B in AFI, or a scale of II or III in WLB underwent biopsies for histopathological examinations. Some areas showing WLB-I and AFI-I also underwent biopsies as negative control.

Results

The mean cigarette index (packs of cigarettes per day × years at that consumption rate) for the 30 smokers was 56 packs (range 3.5–151 packs) and 1 patient was a nonsmoker. The lesions receiving preceding biopsy, radiation therapy, or photodynamic therapy showed AFI-II or AFI-III in spite of their almost normal histology. The same results were seen in LIFE in our previous study. For this reason, we excluded these lesions from the evaluation. Moreover, acute bronchitis showed as a blue image in a wide extent of bronchial mucosa, and the diagnosis of acute bronchitis was facilitated in WLB. These lesions were also excluded in our study. Therefore, 64 lesions were evaluated: 11 lesions were normal, 7 lesions were benign changes including inflammation, fibrosis and hy-

perplasia. Of 29 dysplastic lesions, 16 were of mild, 11 were moderate, and 2 were severe. Seventeen lesions were cancer, and 1 of these lesions was CIS. The relationship between the AFI scale and histopathological diagnosis is shown in table 1.

Sensitivity for severe dysplasia and cancer by WLB-III was 73.7% and that by AFI-III was 94.7%. Sensitivity for moderate dysplasia or worse by WLB-III was 53.5% and that by AFI-III was 76.6%. Sensitivity and specificity with AFI and WLB were summarized in table 2. A typical case is shown in figure 1a and b. It is noted that 3 squamous cell carcinomas showed AFI-III and WLB-II, and detected correctly by AFI (fig. 1c, d). Examination time for AFI and WLB was 15 min on average. There were no adverse events in adding AFI in bronchoscopic examination.

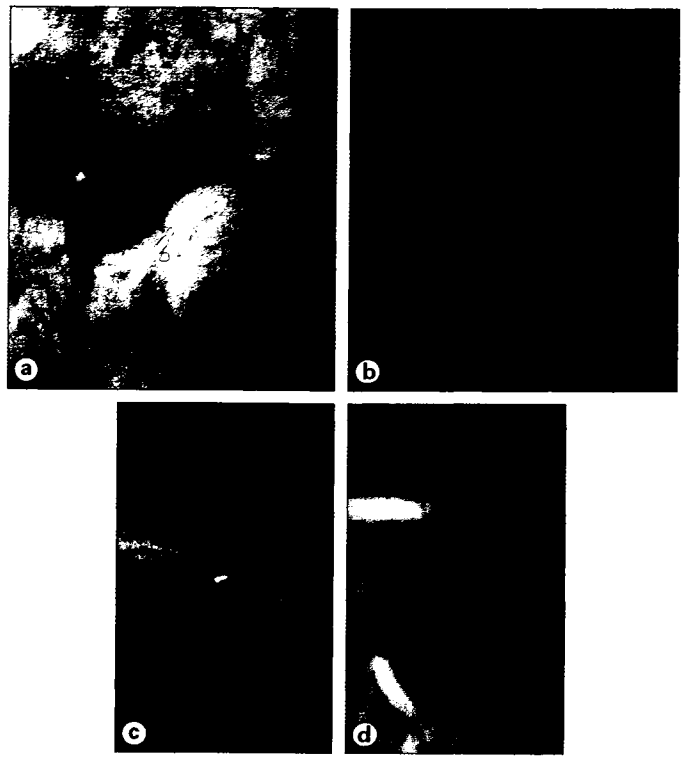

Fig. 1. Squamous cell carcinoma of left B6 (**a** WLB, **b** AFI). Squamous cell carcinoma of right B8+9B10 spur (**c** WLB, **d** AFI).

Table 1. Diagnosis by AFI system and WLB

Histopathology	Biopsy specimens (n = 64)	AFI				WLB		
		AFI-I (n = 19)	AFI-II (n = 11)	AFI-III (n = 31)	AFI-B (n = 3)	WLB-I (n = 17)	WLB-II (n = 17)	WLB-III (n = 18)
Normal + benign changes	18	15	3	0	0	11	7	0
Mild dysplasia	16	2	4	8	2	3	11	2
Moderate dysplasia	11	1	4	5	1	2	7	2
Severe dysplasia	2	1	0	1	0	0	22	0
Cancer	17	0	0	17	0	0	3	14

Table 2. Sensitivity and specificity of AFI and WLB to detect each histopathology

Histopathology	AFI-III		WLB-III	
	sensitivity	specificity	sensitivity	specificity
Moderate dysplasia or worse	76.6%	76.5%	53.3%	94.1%
Severe dysplasia or worse	94.7%	71.1%	73.7%	93.0%
	AFI-II & AFI-III		WLB-II & WLB-III	
	sensitivity	specificity	sensitivity	specificity
Moderate dysplasia or worse	90.0%	50.0%	93.0%	41.2%

Fig. 2. Photomicrograph in the case of AFI-B. Small capillary grows into the moderate dysplastic epithelium. Arrow indicates capillary.

Discussion

Lung cancer is the major cause of cancer death in Japan and most developed countries, and tobacco smoking is one of the causes of this disease. Not only the cessation of smoking, but also early detection and treatment may improve the prognosis of this disease. It is well known that autofluorescence bronchoscopy can improve the detection rate of endobronchial lesions in high-risk groups [8], although Kurie et al. [9] reported that LIFE did not improve the detection of dysplasia or squamous metaplasia. The patient population in Kurie's study may have had a lower possibility of preinvasive cancer than in the other studies.

LIFE, D-light system (Storz, Tuttlingen, Germany), SAFE 1000 autofluorescence system (Pentax, Asahi Optical Tokyo, Japan), and DAFE system (Wolf, Knittlingen, Germany) are commercially available autofluorescence bronchoscopy systems [10–12]. We have been using the LIFE-lung system in clinical practice. LIFE-Lung uses a helium-cadmium laser for excitation at 442 nm, and the emission spectrum is captured by two charge-coupled device cameras and processed through a fluorescence collection sensor and optical multichannel analyzer. Real-time digitized images are acquired by the ratios of red to green (520–630 nm) fluorescence emissions. In comparison, AFI uses a xenon lamp, and lacks a laser generator. AFI afforded clearer images than LIFE because of the utilization of a videoscope system. Moreover, the switch for independent viewing of the two modes of observation reduced the examination time for AFI. In practical use, this switch will be useful because of the need for repeated observations of suspicious areas.

We found the sensitivity of AFI in detection of severe dysplasia and cancer was 94.7%, and the sensitivity of AFI was 21.0% higher than that of WLB. We previously reported that sensitivity of LIFE for detection of severe dysplasia or worse was 83.7% [13]. Sensitivity of LIFE for detection of moderate dysplasia or worse is reported in other literature to lie between 38.0 and 91.0% [14]. In this study, the sensitivity of AFI for detection of moderate or worse was 76.6%. In our study, 3 squamous cell carcinomas diagnosed as moderate dysplasia (WLB-II) in WLB and cancer (AFI-III) in AFI. Haussinger et al. [15] reported that detection of CIS is not increased by autofluorescence bronchoscopy. The reasons for this discrepancy are as follows: sample size of our study was small, and we used a four-point scale in AFI and a three-point scale in WLB, but Haussinger et al. used a two-point scale. Indeed, these lesions were abnormal in both AFI and WLB if we used a two-point scale.

If only WLB-III in WLB is judged as abnormal without considering the AFI finding, 4 lesions with severe dysplasia or worse are missed. These results suggested that AFI has comparable diagnostic ability for detecting precancerous lesion to the other autofluorescence bronchoscopic systems such as LIFE.

Recently, Chiyo et al. [7] compared prospectively AFI with WLB and LIFE. Sensitivity and specificity of AFI for mild, moderate, and severe dysplasia was 80 and 83.3%, respectively. They excluded 2 squamous cell carcinomas which could be detected by all 3 modalities from their analysis; AFI-II and AFI-III in our criteria were judged to be positive in their analysis. In the analysis of our data on the same condition as theirs, sensitivity and specificity were 76.7% (23/30) and 83.0% (15/18), respectively. These results were almost comparable with theirs.

Sensitivity and specificity of the first generation of AFI were not significantly different from those of the second (data not shown). This means that the diagnostic ability of AFI is not dependent upon the order of bronchoscopic procedure. Hirsch et al.'s [14] report also showed that there was no difference in the sensitivity and specificity, regardless of the order of the bronchoscopic procedure.

Tracheobronchial mucosa that contains a large amount of blood should be indicated by blue images with AFI. The extended vessels in mucosa and any bleeding area are actually shown in blue. Most of the lesions that exhibited blue in AFI were inflammation unless they were bleeding. However, three blue lesions in AFI were mild or moderate dysplasias (fig. 2). The reason for this is not clear at present. Angiogenic squamous dysplasia (ASD) is a newly recognized morphological entity. ASD consists of capillary blood vessels closely juxtaposed to and pro-

jected into metaplastic or dysplastic squamous epithelium [16]. Keith et al. [16] reported that ASD was observed in many high-risk smokers and patients with squamous cell cancer and may represent an important intermediate pathological biomarker preceding lung cancer development. Two of three blue lesions showed an increased number of microcapillaries and this might result in increasing blood supply in the mucosa; an increase in the blood supply results in an increase in the blue signal. LIFE detected more ASD lesions than WLB. Regarding hemoglobin content, AFI has more information than LIFE because of adding R'-image information. AFI may be useful for detecting ASD.

We conclude that AFI is a useful system to detect cancer and precancerous lesions, and is used for screening of the high-risk group. Since the number of severe dysplasias and CIS was small in our study, multicenter trials will be necessary to confirm this conclusion.

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INTERNAL MEDICINE

□ PICTURES IN CLINICAL MEDICINE □

Metastatic Serous Adenocarcinoma Arising in the Adnexa Uteri and Forming Pleural Cysts on the Diaphragmatic Pleura

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Key words: pleural cyst, serous adenocarcinoma, adnexa uteri, pleural metastasis

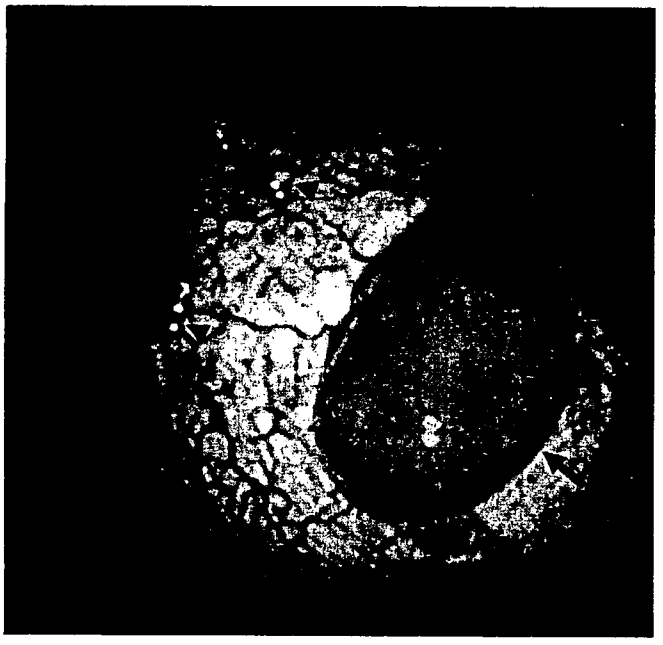

Figure 1. **Left thoracoscopy showed a pleural cyst measuring 1.5cm in diameter (arrow) and adjacent daughter cysts (arrowheads) on the diaphragmatic pleura.**

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A 74-year-old woman consulted our hospital complaining of cough that had persisted for the previous 3 months. Chest computed tomographic (CT) scan showed bilateral pleural effusion without any pulmonary lesions. Pleural effusion cytology showed adenocarcinoma. Barium enema, gastroduodenoscopy and abdominal CT did not demonstrate any abnormal findings. Serum CEA, NSE and CYFRA21-1 were 26.8 (cutoff: 5) ng/ml, 43.7 (cutoff: 10) ng/ml and 67.5 (cutoff: 3.5) ng/ml, respectively. After removal of 1,500 ml of pleural effusion, left thoracoscopy showed a few eccentric pleural cysts on the diaphragmatic pleura (Fig. 1). No pleural nodule suggestive of malignancy was recognized. The content of the cyst was clearly serous fluid. Pathologic examination of the cyst showed a small focus of adenocarci-

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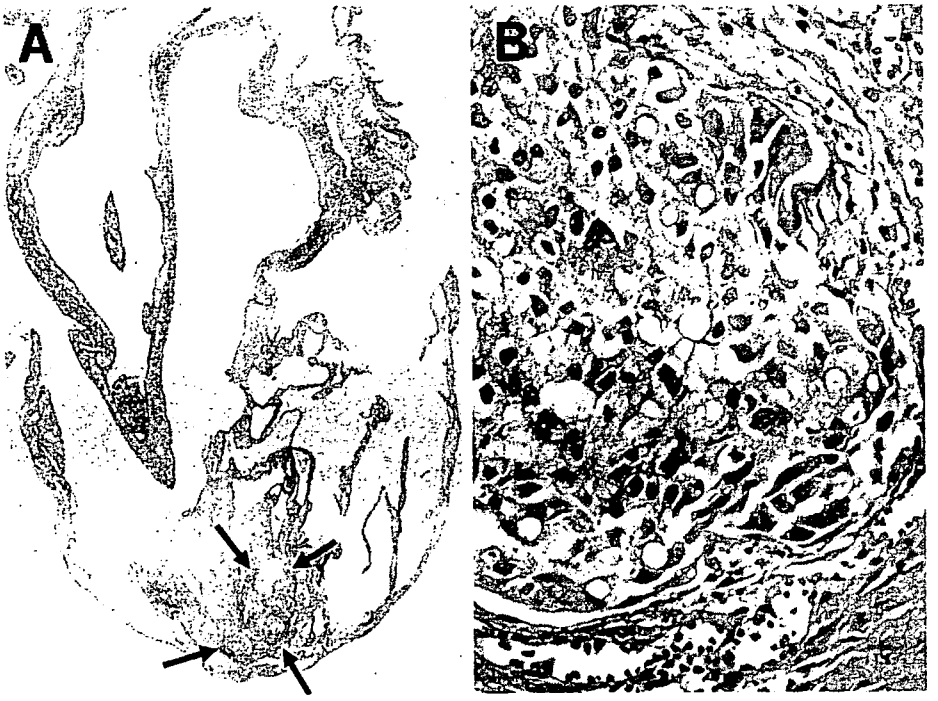

Figure 2. **A: Microscopically, the pleural cyst was unilocular. A small focus of adenocarcinoma was recognized in the cyst wall (arrows). B: Most tumor cells had abundant clear or pale eosinophilic cytoplasm, oval nuclei and inconspicuous nucleoli. Stain: hematoxylin and eosin; magnification A: ×2.5, B: ×100.**

noma (Fig. 2a, b). Immunohistochemical studies showed that these carcinoma cells were positive for AE1/AE3, EMA, CA125 and cytokeratin (CK)-7, but negative for CEA, TTF-1 and CK-20. The tentative diagnosis was Stage IV pulmonary adenocarcinoma. Systemic chemotherapy achieved stable disease. Six months later, the patient underwent surgery for right uterine adnexal tumor with diffuse peritoneal dissemination. Pathologic examination of the resected specimen demonstrated that the tumor was a poorly differentiated serous adenocarcinoma arising in the right adnexa uteri. Conclusively, we diagnosed pleural lesions as distant metastases of uterine adnexal serous adenocarcinoma. To our knowledge, the formation of these pleural cysts by metastatic carcinoma has not yet been reported in the literature. We propose two possible explanations for cyst formation by metastatic lesions: 1) localized edema in the submesothelial space due to carcinomatous obstruction of superficial vessels in the pleura caused pleural cysts; and 2) metastatic cancer cells in the pleura produced serous fluid in the submesothelial space and formed cystic lesions. The elucidation of its etiology, however, requires the accumulation of additional cases. Thoracic oncologists and pathologists should be aware of the varied gross manifestations of metastatic adenocarcinoma to the pleura and should bear in mind the differential diagnoses of pleural cysts.

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Letter to the Editor

Pleural sarcomatoid malignant mesothelioma consisting of histiocytoid cells

To the Editor:

Pleural sarcomatoid malignant mesothelioma (PSMM) consists of spindle cells arranged in fascicles or having a haphazard fashion. The pattern often resembles fibrosarcoma, but marked anaplasia and bizarre multinucleated tumor cells may result in a picture closely mimicking that of malignant fibrous histiocytoma.[1] We herein report an extremely rare case of PSMM consisting of histiocytoid cells without any inflammatory infiltrate. We discuss its differential diagnosis and the key to establishing an accurate diagnosis.

A 76-year-old Japanese woman, a non-smoker, consulted Osaka Prefectural Medical Center for Respiratory and Allergic Diseases complaining of left chest pain that had persisted for the previous 2 weeks. She had worked as a spinning-mill worker for more than 30 years and occupational exposure to asbestos was highly supported. She had a past history of pleuritis and pericarditis of unknown origin. Chest X-ray demonstrated two fist-sized subpleural nodules in the left mediastinal and parietal costal pleura without any pulmonary lesions. There was a large amount of bloody pleural effusion on the left side. Pleural effusion cytology did not demonstrate any neoplastic findings. The course of the disease was rapidly progressive. A tentative clinical diagnosis of pleuritis carcinomatosa was made and percutaneous needle biopsies were obtained from the subpleural lesions. Histologically, most tumor cells infiltrated into a myxoid stroma and grew as a diffuse pattern (Fig. 1a). Sheets and nests of cells were absent. Inflammatory infiltrate was slight. The tumor cells were mainly composed of histiocytoid cells (Fig. 1b). These histiocytoid cells were ovoid or polyhedral in shape, with palely eosinophilic or foamy cytoplasm. Their nuclei were medium to large, ovoid or angulated, contained fine chromatin, and inconspicuous nucleoli (Fig. 1c). Multinucleated giant tumor cells were rarely recognized. Mitoses were 2/10 high-power fields (HPF). Necrosis and hemorrhagic areas were absent. Reactive histiocytes were sometimes present among these neoplastic cells. Immunohistochemical studies showed that these tumor cells were positive for AE1/AE3 (Fig. 2a), D2-40 (Fig. 2b), HBME-1, vimentin and WT-1 (Fig. 2c), but negative for BerEP4, CA125, calretinin, CAM5.2, CD3, CD15, CD30, CD34, CD45RB, CD79α, CEA, cytokeratin 5/6, cytokeratin 7, desmin, epithelial membrane antigen, napsin A, PE-10, PG-M1, S-100, Smooth-muscle actin, thrombomodulin and thyroid transcription factor-1. MIB-1 was positive in 20% of tumor cells. PAS reaction was positive in some tumor cells and was partly digested with diastase. Alcian blue stain was positive in stroma digested with hyaluronidase and negative in the cytoplasm. Colloidal iron stain was positive in the stroma and digested with hyaluronidase. Taken together, we made a diagnosis of pleural sarcomatoid malignant mesothelioma consisting of histiocytoid cells.

Recognition of this rare variant of malignant mesothelioma is important because of the possibility of confusing it with lymphohistiocytic variant of anaplastic large cell lymphoma (LHALCL), sarcomatoid carcinoma (SC) of the lung or thymus, inflammatory malignant fibrous histiocytoma (IMFH), inflammatory myofibroblastic tumor (IMT) or lymphohistiocytoid mesothelioma (LHM). In LHALCL, large-sized atypical cells with immunohistochemical CD30 positivity are admixed with histiocytes and plasma cells.[2] However, in this tumor, immunohistochemical studies showed that the atypical cells were negative for CD30. As for SC, lesions of the lung and anterior mediastinum were absent, and spindle or giant atypical cells were not apparent histologically in the present case. IMFH is composed of xanthogranulomatous inflammation with scattered atypical large cells with prominent nucleoli. The present tumor lacked xanthogranulomatous inflammation, and histiocytoid cells were not so large and nucleoli were inconspicuous. IMT is composed of a variable mixture of collagen, inflammatory cells and cytologically bland spindle cells having myofibroblastic differentiation. However, in the present case, collagen and these bland spindle cells were absent. LHM is a variant of sarcomatoid malignant mesothelioma, characterized by diffuse proliferation of large, ovoid histiocyte-like and spindle cells, uniformly intermixed with a prominent lymphocytic or lymphoplasmacytic infiltrate.[3,4] But in the present case the lymphocytic or lymphoplasmacytic infiltrate was subtle.

We made a diagnosis of PSMM consisting of histiocytoid cells based on percutaneous needle biopsy specimens. The keys to accurate diagnosis were as follows: (i) recognition of serosal tumor; (ii) immunohistochemical positivity for AE1/AE3, vimentin and mesothelial markers including D2-40,[5] WT1 and HBME-1; and (iii) being aware of the varied histopathological manifestations of PSMM.

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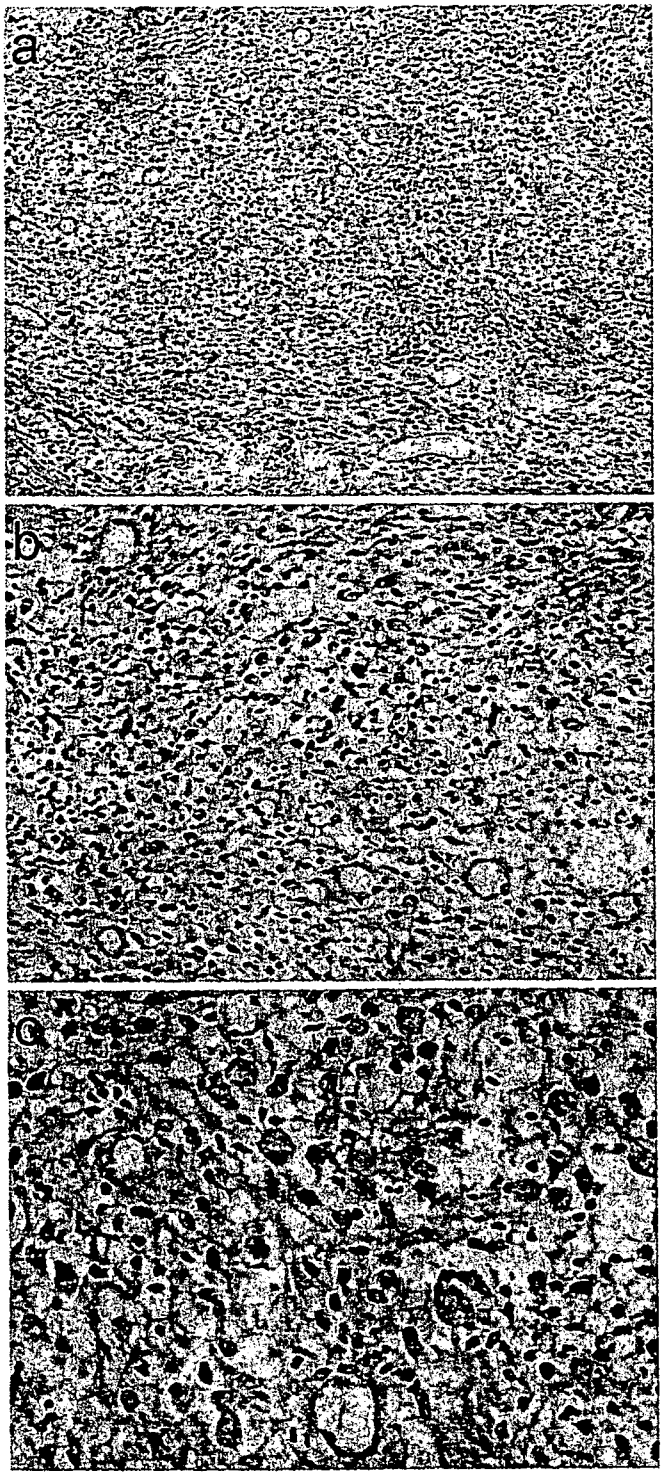

Figure 1 Microscopic view of the tumor. (a) Tumor cells infiltrating diffusely in a myxoid stroma. (b) Tumor cells mainly composed of histiocytoid cells. (c) The tumor cells were ovoid or polyhedral in shape with palely eosinophilic or foamy cytoplasm. Stain: HE.

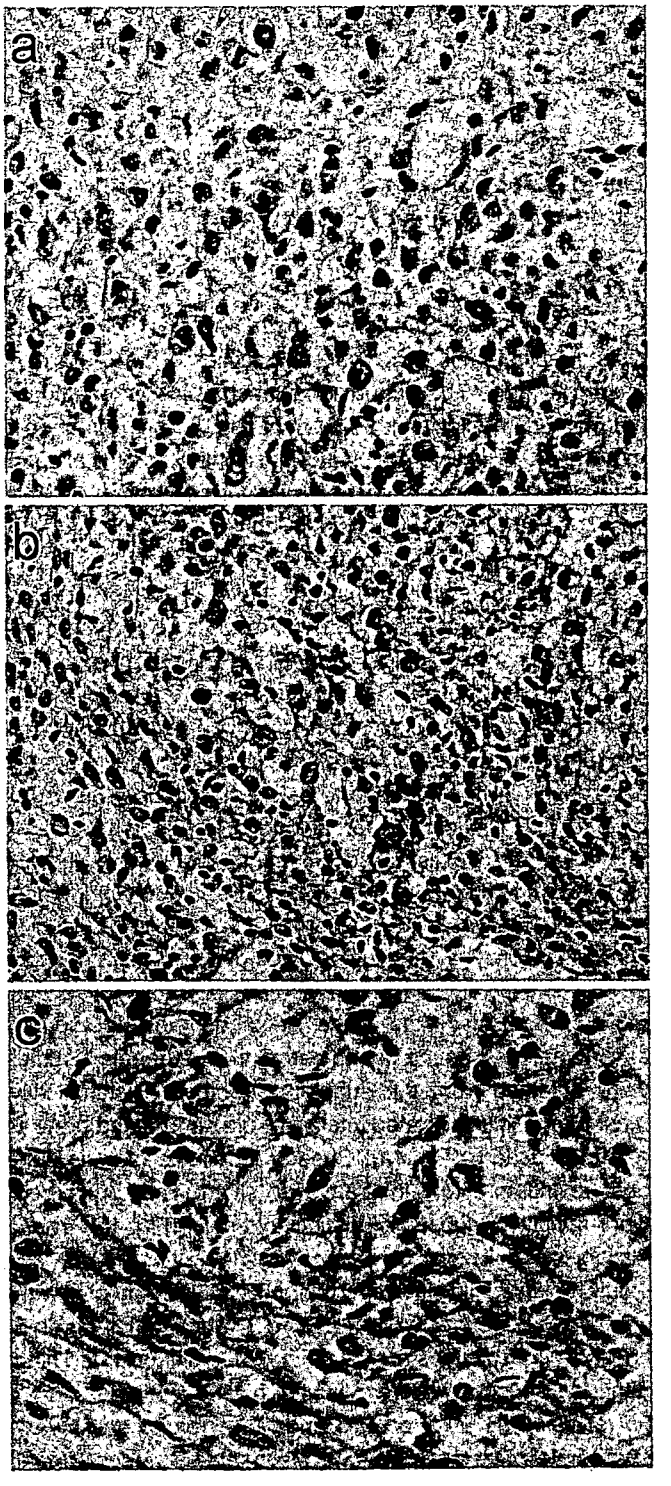

Figure 2 Immunohistochemical staining of the tumor. The histiocytoid cells had positive staining for (a) AE1/AE3, (b) D2-40 and (c) WT1.

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ORIGINAL ARTICLE

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Preliminary experience with a modified premedication protocol that included intravenous diphenhydramine and calcium bromide for the prophylaxis of paclitaxel-related hypersensitivity reactions

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Abstract

Background. Paclitaxel often causes severe hypersensitivity reactions (HSRs) rapidly after infusion, even in patients given prophylactic therapy. The purpose of this study was to analyze the incidence of paclitaxel-related HSRs in patients with non-small cell lung cancer (NSCLC) retrospectively, and to assess the feasibility of a modified premedication protocol.

Methods. One hundred and seven patients who were pretreated with either a conventional premedication regimen (two doses of dexamethasone) or a short premedication regimen (single dose of dexamethasone with oral diphenhydramine and intravenous ranitidine), prior to paclitaxel infusion were retrospectively analyzed. A modified premedication regimen, consisting of 12.5 ml of Rescalmin (intravenous diphenhydramine 50 mg and calcium bromide 437.5 mg), intravenous ranitidine 100 mg, and intravenous dexamethasone 20 mg, was given 30 min prior to paclitaxel, with oral dexamethasone 8 mg given on the night before the paclitaxel. Patients received paclitaxel intravenously at 175 mg/m^2 over 3 h, followed by carboplatin, AUC 5, over 1 h on day 1 every 3 weeks.

Results. In the conventional premedication group, 21 patients had HSRs (32.3%); in 1 of these patients the HSR was considered to be severe (1.5%). In the short premedication group, 19 patients had HSRs (45.2%); in 6 of these patients the HSRs were considered to be severe (14.3%). The incidence of severe HSRs was significantly higher in the short premedication group than in the conventional premedication group ($P = 0.027$). In the modified premedication protocol study, HSR events were recorded in 14 patients (63.6%); 14 showed flushing, 2 had skin rash, and 1 had tachycardia. No severe HSRs were seen.

Conclusions. The incidence of HSRs in the short premedication group tended to be higher than that in the conventional premedication group. The modified premedication protocol was found to be feasible for preventing paclitaxel-related HSR, but case accumulation is needed.

Key words Paclitaxel · Premedication · Hypersensitivity reactions · Prophylaxis · Diphenhydramine

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Introduction

Paclitaxel is a highly active drug used for the treatment of lung, ovarian, breast, head and neck, bladder, and other epithelial cancers. In early phase I trials, a high frequency of severe hypersensitivity reactions (HSRs) was observed when paclitaxel was administered.[1–3] HSRs usually occur just after the start of paclitaxel administration. The reaction likely occurs due to the release of histamine and other vasoactive compounds from mast cells in response to the polyoxyethylated castor oil vehicle (Cremophor El, Sigma Chemical Co., St. Louis, MO).[4] Severe HSRs, characterized by chest pain, dyspnea, bronchospasm, urticaria, and/or hypotension were initially reported in 10.6% of patients who were not premedicated prior to paclitaxel infusion.[4] After the initial report of HSR events to the National Cancer Institute, it was recommended that all patients receiving paclitaxel be given conventional premedication, which contained two doses of oral dexamethasone, intravenous diphenhydramine, and intravenous cimetidine or ranitidine.[5] Consequently, the incidence of severe HSRs has decreased to 1%–3%.[6–8] It has become common practice to pretreat patients with various regimens prior to paclitaxel administration.

Currently, the standard recommended prophylactic therapy regimen is a single dose of intravenous dexamethasone, intravenous diphenhydramine, and intravenous cimetidine or ranitidine,[8–11] which is called a short premedication regimen. However, it has been reported that short premedication may not be the optimum prophylactic therapy for paclitaxel-related HSRs.[12,13] In Japan, oral diphenhydramine has usually been used as a prophylactic H1 antagonist, because pure intravenous diphenhydramine has not been not available. We hypothesized that the blood concentration of diphenhydramine when the oral form is used may be more influenced by the patient's condition (for example, by the presence of gastrointestinal disease, or advanced age) than when the intravenous form is used.

In this study, we retrospectively analyzed the incidence of paclitaxel-related HSRs in patients with non-small cell lung cancer (NSCLC). Also, we carried out prophylactic treatment with a modified premedication protocol, using Rescalmin (diphenhydramine with calcium bromide; Nissin, Yamagata, Japan) – a product which is usually used intravenously for allergic rhinitis – to assess its feasibility for preventing paclitaxel-related HSRs.

Patients and methods

Definition of paclitaxel-related hypersensitivity reactions

Reactions were scored as "severe" if, during paclitaxel infusion, the patient experienced one or more of the following grade 3–4 toxicities: angioderma, chest and/or back pain, dyspnea and/or wheezing, hypotension requiring vasopressor agent support, or cardiac arrest. If patients with a grade 2 HSR, such as chest or back pain, strongly desired to stop the infusion, we classified the HSR as severe in such patients. Reactions were scored as mild if one of the following grade 1–2 toxicities was recorded: flushing, mild hypotension, skin rash, or palpitation.

Retrospective historical cohort analysis of paclitaxel-related hypersensitivity reactions

We retrospectively analyzed the incidence of paclitaxel-related HSRs in patients with NSCLC. A pharmacy database at Osaka Prefectural Medical Center for Respiratory and Allergic Diseases identified all patients who had received paclitaxel with either conventional or short premedication from April 1999 to March 2002 (Table 1). All the patients had received both H1 and H2 antagonists (50 mg oral diphenhydramine and 100 mg intravenous ranitidine) 30 min prior to the paclitaxel infusion. In addition, the conventional premedication group had received two 20 mg doses of intravenous dexamethasone, at 12 and 6 h prior to the paclitaxel. The short premedication group had received a single 20-mg dose of intravenous dexamethasone 30 min prior to the paclitaxel. Paclitaxel was administered at a dose of 175–200 mg/m^2 by infusion over 3 h.

Treatment evaluations consisted of a complete medical history and physical examination, which included a blood cell count, urinalysis, ECG, chest X-ray, bone scan, and computed tomography. HSRs were graded according to the National Cancer Institute common toxicity criteria (NCI-CTC version 2.0; January 30, 1998) for adverse reactions to chemotherapy. Statistical significance was calculated with the Yate's corrected χ^2 statistic. A difference with a P value of less than 0.05 was considered to be significant. Statistical analysis software (StatMate III, ATMS, Tokyo, Japan) was used for the analysis.

Table 1. Premedication details and characteristics in patients receiving paclitaxel

	Total	Conventional	Short
Total no. of patients	107	65	42
Sex			
Male	77	48	29
Female	30	17	13
Median age, years (range)	61 (32–75)	61 (32–75)	62.5 (34–74)
Performance status			
0–1	83	50	33
2–3	24	15	9
Histological type			
Adenocarcinoma	84	52	32
Squamous cell carcinoma	19	10	9
Large cell carcinoma	4	3	1
Prior chemotherapy			
Yes	25	13	12
No	82	52	30
Allergic history			
Yes	5	2	3
No	102	63	39

Modified premedication protocol for prophylaxis of paclitaxel-related hypersensitivity reactions

We conducted a prospective trial to assess the feasibility of using a modified premedication protocol for the prophylaxis of paclitaxel-related HSRs. To be eligible, patients had to have histologically or pathologically documented NSCLC. Measurable disease was not necessary. Patients were required to have, at study entry, an Eastern Cooperative Oncology Group (ECOG) performance score of 0 to 2, and were required to have an absolute neutrophil count of 2000/μl or more, a platelet count of 100 000/μl or more, a WBC count of 3500/μl or more, and a hemoglobin level of 9.5 g/dl or more. The total bilirubin level was required to be less than 1.5 times the upper normal limit. The serum creatinine level was required to be less than the upper normal limit. Patients were required to have recovered from toxicities of prior chemotherapy, and may not have had either radiation therapy or investigational drug therapy within 4 weeks of initiating paclitaxel and carboplatin. This protocol was reviewed and approved by the institutional review board, and all patients gave written informed consent before participation.

All patients received 12.5 ml of Rescalmin (50 mg intravenous diphenhydramine with 437.5 mg calcium bromide; Nissin), 100 mg intravenous ranitidine, and 20 mg intravenous dexamethasone, 30 min prior to the paclitaxel infusion,

after having oral dexamethasone 8 mg the night before the paclitaxel. Paclitaxel was administered intravenously at 175 mg/m^2 over 3 h, followed by carboplatin, AUC 5, over 1 h on day 1 every 3 weeks. The calculated dose of paclitaxel was diluted in 500 ml of 5% dextrose in water. Polyolefin containers and polyethylene-lined tubing were used for drug administration because of concern that the vehicle in which paclitaxel was prepared, Cremophor EL, might leach plasticizer from polyvinylchloride-containing intravenous sets.

During the infusion, patients' vital signs (heart rate, respiratory rate, and blood pressure) were determined every 15 min for the first hour, and every 30 min for the next 2 h. Continuous cardiac monitoring was required until 6 h after the completion of the paclitaxel infusion.

Treatment cycles were repeated every 3 weeks, provided toxic effects were not prohibitive and there was no evidence of tumor progression. Doses were to be reduced in the event of treatment-related febrile neutropenia, grade 4 neutropenia, or grade 3 nonhematological toxicity. Paclitaxel was discontinued if there was more than grade 2 neurologic toxicity, cardiac arrhythmias, heart block, or a significant HSR. Minor reactions were to be managed by stopping the infusion, if judged medically necessary, and by administering symptomatic medications such as additional antihistamines, corticosteroids, or bronchodilators.

Results

Retrospective historical cohort analysis

One hundred and seven patients were identified in the database. Up to November 2000, 65 patients had received the conventional prophylactic regimen, and from December 2000, 42 patients had received the short prophylactic regimen. Table 2 shows the incidence of HSRs in the two prophylactic regimens. In the conventional premedication group, 21 patients had HSRs (32.3%); in 1 of these patients, the HSR was considered to be severe (1.5%). In the short premedication group, 19 patients had HSRs (45.2%); in 6 of these patients, the HSRs were considered to be severe (14.3%). In this historical cohort analysis, the overall incidence of HSRs in the short premedication group was not significantly different from that in the conventional premedication group (χ^2; $P = 0.177$), but the incidence of severe HSRs was found to be significantly higher in the short premedication group (χ^2; $P = 0.027$). Table 3 shows a summary of the severe HSRs in the 7 patients. In the 6 patients in the short premedication group, the hypersensitivity events occurred soon after the paclitaxel was initiated in the second course, and the reactions included chest or back pain, and dyspnea with or without bronchospasm. In the 1 patient in the conventional premedication group, grade 3 dyspnea with bronchospasm occurred during the first course. Paclitaxel infusion was discontinued immediately in all the patients with severe HSRs, and they received corticosteroid treatment. None of the patients were in a critical state, and there were no treatment-related deaths.

Modified premedication regimen experience

Patients

From January 2004 to May 2004, 22 patients were enrolled in this study (Table 4). The patients were predominantly male (20 of 22 patients), and the median age was 65 years (range, 38 to 74 years). Nineteen (86.4%) of the 22 patients had an ECOG performance status of 0 or 1, 8 (36.4%) had metastatic lesions (stage IV), 17 (77.3%) had adenocarcinoma, and 7 (31.9%) had had prior chemotherapy.

Adverse events

Toxicity data were available for all 22 patients who had received at least one dose of paclitaxel. Overall, the therapy was generally well tolerated and manageable. The patients' nonhematological toxicities are listed in Table 5. In this study, HSRs were recorded in 14 patients (63.6%); 14 showed flushing (grade 1), 2 had skin rash (1 of grade 1 and 1 of grade 2), and 1 had tachycardia (grade 1). No severe

Table 2. Comparison of incidence of hypersensitivity reactions (HSRs) with the two prophylactic regimens

	Conventional	Short	*P* value
Overall HSR			0.177
(+)	21 (32.3%)	19 (45.2%)	
(−)	44 (67.7%)	23 (54.8%)	
Severe HSR			0.027
(+)	1 (1.5%)	6 (14.3%)	
(−)	64 (98.5%)	36 (85.7%)	

Table 3. Summary of severe hypersensitivity reactions

Patient no.	Age (years)	Sex	Premedication	Symptoms	Onset[a]	Course	NCI-CTC
1	71	M	Short	Back pain	Soon	2	2
2	70	F	Short	Angioderma, dyspnea without bronchospasm	5 min	2	3
3	64	F	Short	Chest pain, back pain, flushing	Soon	2	2
4	52	F	Short	Chest pain, back pain, flushing	Soon	2	2
5	71	M	Short	Dyspnea with bronchospasm	10 min	2	3
6	34	M	Short	Dyspnea with bronchospasm	Soon	2	3
7	69	M	Conventional	Dyspnea with bronchospasm	10 min	1	3

[a] In relation to paclitaxel infusion

Table 4. Characteristics of patients with modified premedication protocol ($n = 22$)

Total no. of patients	22
Sex	
Male	20
Female	2
Median age, years (range)	65 (38–74)
Performance status	
0–1	19
2	3
Stage	
IIIA	8
IIIB	6
IV	8
Histological type	
Adenocarcinoma	17
Squamous cell carcinoma	2
Large cell carcinoma	3
Chemotherapy	
First-line	15
Second-line	6
Third-line	1

Table 5. Nonhematological toxicity observed in patients with modified premedication protocol ($n = 22$)

	Grade (no. of patients)							
	1	(%)	2	(%)	3	(%)	4	(%)
Nausea	11	(50)	1	(4)	1	(4)	–	
Vomiting	1	(4)	0	(0)	1	(4)	0	(0)
Appetite loss	12	(54)	1	(4)	2	(9)	0	(0)
Neuropathy	8	(36)	3	(13)	0	(0)	0	(0)
Myalgia	5	(22)	6	(27)	0	(0)	0	(0)
Arthralgia	6	(27)	8	(36)	0	(0)	0	(0)
Diarrhea	1	(4)	0	(0)	1	(4)	0	(0)
Alopecia	2	(9)	9	(40)	–	–		
Fatigue	14	(63)	2	(9)	1	(4)	0	(0)
Somnolence	11	(50)	0	(0)	0	(0)	0	(0)
Hypersensitivity reactions								
Flushing	14	(63)	0	(0)	0	(0)	0	(0)
Tachycardia[a]	1	(4)	0	(0)	0	(0)	0	(0)
Skin rash[a]	1	(4)	1	(4)	0	(0)	0	(0)

Toxicity assessed according to NCI-CTC version 2
[a]Three patients also showed flushing

HSRs were seen. All adverse events resolved naturally without corticosteroid administration. Other nonhematological toxicities, some of which were grade 3, were mainly digestive toxicities, such as appetite loss, vomiting, constipation, and diarrhea. In addition, 11 (50%) of the 22 patients had mild somnolence, which symptom disappeared immediately after the end of treatment.

Drug delivery

Overall, the median cumulative paclitaxel exposure of the 22 patients was 665 mg/m^2 (range, 175–1050 mg/m^2). The average number of cycles delivered was 3.5 (range, 1–6). The dose was reduced in 18% of the patients because of hematological toxicity.

Discussion

Although paclitaxel–based chemotherapy is widely used for patients with NSCLC,[14] severe HSRs are reported more frequently with paclitaxel treatment than with other cytotoxic chemotherapeutic drugs. If severe HSRs occur, the paclitaxel treatment is discontinued. This is a disadvantage for patients, so prophylactic treatment has been used.[4–7] Dexamethasone is a long-acting glucocorticoid with a biologic half-time of approximately 48 h.[15] Currently, a short premedication regimen including single-dose intravenous dexamethasone has been recommended.[8–11] A comparative prospective study has reported that the incidence of paclitaxel-related HSRs was not significantly different between conventional and short premedication regimens.[14] However, Kwon et al.[12] retrospectively showed that a single-dose intravenous corticosteroid prophylactic regimen was associated with a significantly higher rate of HSRs than the two-dose oral corticosteroid regimen. Moreover, Kloover et al.[13] have reported that short premedication may not be a suitable prophylactic therapy for paclitaxel-related HSR because of a fatal outcome.

We retrospectively analyzed, in a historical cohort, the incidence of paclitaxel-related HSRs in patients who had received oral diphenhydramine, plus a single dose or two doses of intravenous dexamethasone. We found that six of the patients with a short premedication regimen had severe HSRs, which events occurred as soon as paclitaxel was initiated in the second course. These events included chest or back pain, hypoxia, dyspnea, and bronchospasm, and the incidence of severe HSRs was significantly higher than that in the conventional premedication group. Since obtaining the results of the historical analysis, we immediately stopped using the short premedication regimen. The incidence of severe HSRs in the patients in the short premedication group was quite high compared with that in past reports. Possibly, our definition of severe HSR may have differed from that used previously. However, grade 2 chest or back pain should be considered as severe, and paclitaxel infusion should be stopped for safety, because such symptoms have a possibility to lead to serious toxicity, such as cardiac arrest.[13]

In Japan, oral diphenhydramine had usually been used as a prophylactic H1 antagonist, because pure intravenous diphenhydramine was not available. With the oral product, the blood concentration is thought to be more influenced by the patient's general condition (for example, by the presence of gastrointestinal disease, or advanced age) than when the intravenous form is used:. Bearing in mind its pharmacological properties, oral diphenhydramine plus single-dose intravenous dexamethasone is unlikely to result in an adequate level of immunosuppression during the infusion of paclitaxel. This may explain the results of our historical analysis. As a result of these concerns, we employed a modified premedication protocol, using Rescalmin (Nissin) intravenously instead of oral diphenhydramine, with a dose of oral dexamethasone being administered the night before the paclitaxel infusion.

Paclitaxel treatment using the modified premedication protocol was performed smoothly and good compliance was obtained. There were no severe HSRs, and no treatment was discontinued because of toxic allergic reactions. This treatment regimen seems to be effective for the prophylaxis of paclitaxel-related HSRs, although the number of patients in our study was small. This treatment regimen has several advantages, as follows. First, it ensures that an intravenous H1 antagonist is administered prior to paclitaxel, in contrast to the administration of the oral product. Second, because the dose of oral dexamethasone given the night before the paclitaxel infusion is lower than the conventional dose, patients' compliance is better. Third, mild somnolence seems to be a favorable effect of receiving chemotherapy in anxious patients. Finally, this treatment regimen might be useful not only for paclitaxel but also for other chemotherapeutic drugs such as docetaxel, oxaliplatin, and cetuximab. In fact, in a patient with docetaxel-related HSR, re-administration of docetaxel succeeded with the modified premedication protocol. Our modified protocol might also be useful with oxaliplatin, a platinum salt which is particularly effective in treating colorectal cancer, but with which, as a result of its increasing clinical use, a rising incidence of HSRs has been observed.[16] HSRs have also been observed with cetuximab, a monoclonal antibody that is an inhibitor of epidermal growth factor receptor.[17]

In conclusion, in our historical cohort analysis, the incidence of HSRs in the short premedication group tended to be higher than that in the conventional premedication group. Our modified premedication protocol was found to be feasible for preventing paclitaxel-related HSRs, but case accumulation is needed.

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Proteomics-based identification of α-enolase as a tumor antigen in non-small cell lung cancer

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Autoantibodies against tumor antigens represent one type of biomarker that may be assayed in serum for detection of cancer and monitoring of disease progression. In the present study, we used a proteomics-based approach to identify novel tumor antigens in non-small cell lung cancer (NSCLC). By combining two-dimensional electrophoresis, western blotting, mass spectrometry and enzyme-linked immunosorbent assay technology, we detected autoantibodies against α-enolase in a subset of NSCLC patients' sera. When 'Mean $OD_{\text{healthy control sera}}$ + 3 $SD_{\text{healthy control sera}}$' was used as the cut-off point, the prevalence of this autoantibody was 27.7% in patients with NSCLC (26 of 94), 1.7% in healthy control subjects (1 of 60), and not detectable in sera from 15 patients with small cell lung cancer, 18 patients with gastrointestinal cancer and nine patients with *Mycobacterium avium* complex infection of lung. Immunohistochemical staining showed that expression of α-enolase was increased in cancer tissues of NSCLC patients, and flow cytometric analysis confirmed the expression of α-enolase at the surface of cancer cells. The combined detection of autoantibodies against α-enolase, carcinoembryonic antigen and cytokeratin 19 fragment (CYFRA21-1) enhanced sensitivity for the diagnosis of NSCLC. Therefore, autoantibodies against α-enolase may constitute a promising biomarker for NSCLC. (*Cancer Sci* 2007; 98: 1234–1240)

Lung cancer is the leading cause of cancer death,[1] and NSCLC accounts for nearly 80% of lung cancer cases. There is an urgent need for a better understanding of the biological mechanisms of NSCLC as well as the identification of reliable biomarkers for its diagnosis and prognosis. To date, a number of NSCLC markers have been evaluated, including CEA, CYFRA 21-1, SCC antigen, CA125 and NSE.[2–8] Autoantibodies against several tumor antigens such as L-myc and c-myc, p53 and antinuclear/antinuclear antigens have also been investigated.[9–12] Recently, autoantibodies against PGP9.5, peroxiredoxin-I, annexin-I and annexin-II were identified in the sera of lung cancer patients using a proteomic approach.[13–15] However, the sensitivity and specificity of these biomarkers are not yet satisfactory and there are currently no data to support any particular method for screening for lung cancer.[16]

Autoantibodies against tumor antigens represent one type of biomarker that may be assayed in serum for detection of cancer and monitoring of disease progression. In spite of the fact that the quantity of any tumor antigen in cancer cells or in the circulation is usually very small, especially in the early stages of cancer, the body's immune response to such antigens represents a remarkable phenomenon of biological amplification of these weak signals from tumor antigens.[17] The identification of panels of tumor antigens that elicit an immune response may thus be useful for detecting potential specific biomarkers as well as for the initiation of immunotherapy against NSCLC. The aim of the present study was to identify novel candidate tumor antigens in NSCLC by means of a proteomics-based approach. One of these antigens was identified as α-enolase, and its immunogenicity was confirmed by western blotting using recombinant protein. The results obtained with enzyme-linked immunosorbent assay (ELISA) demonstrated that when 'Mean $OD_{\text{healthy control sera}}$ + 3 $SD_{\text{healthy control sera}}$' was used as the cut-off point, a humoral immune response directed against α-enolase occurred in 27.7% of NSCLC patients, but in only 1.7% of healthy control subjects. Immunohistochemical staining showed that α-enolase was overexpressed in cancer tissues of NSCLC patients. The combined detection of autoantibodies against α-enolase, CEA and CYFRA 21-1 enhanced sensitivity for NSCLC diagnosis. Therefore, autoantibodies against α-enolase may constitute a promising biomarker for NSCLC.

Materials and Methods

Subjects. Sera and tumor tissue were obtained at the time of diagnosis after informed consent had been given by the subjects. The experimental protocol was approved by the ethics committee of Osaka University. Sera from 94 patients with NSCLC, 15 patients with SCLC, 18 patients with gastrointestinal cancer (10 patients with gastric cancer, 8 patients with colon cancer) and nine patients with MAC were analyzed. In terms of TNM stages, the NSCLC patients comprised 17 cases of stage I, 14 cases of stage II, 34 cases of stage III and 29 cases of stage IV. The histological distribution of NSCLC was 73 adenocarcinoma cases and 21 SCC cases. Clinical data for the serum tumor marker CEA and CYFRA 21-1 were also collected for investigation. Sera from 60 asymptomatic healthy subjects, whose average age and sex were comparable to those of the NSCLC patient group, were used as controls.

1-DE and 2-DE. Proteins were extracted from NSCLC tumor tissues using the Complete Mammalian Proteome Extraction Kit (Calbiochem, Darmstadt, Germany). For 1-DE, extracted proteins

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Abbreviations: 1-DE, one-dimensional electrophoresis; 2-DE, two-dimensional electrophoresis; a.a., amino acids; CA, cancer antigen; CBB, Coomassie brilliant blue; CEA, carcinoembryonic antigen; CYFRA21-1, cytokeratin 19 fragment; ELISA, enzyme-linked immunosorbent assay; HRP, horseradish peroxidase; IEF, isoelectric focusing; IHC, immunohistochemical; LC, liquid chromotography; MAC, Mycobacterium avium complex; MALDI-TOF, matrix-assisted laser desorption–ionization time-of-flight; MS, mass spectrometry; NSCLC, non-small cell lung cancer; NSE, neuronspecific enolase; OD, optical density; PCR, polymerase chain reaction; PMF, peptide mass fingerprinting; PVDF, polyvinylidene difluoride; SCC, squamous cell carcinoma; SCLC, small cell lung cancer; TNM, tumour–node–metastasis.

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