合でも，ポンプへ過剰圧がかかれば同様のケースが発生する危険があり，類似のペリスタルティック方式を採用しているCADD™-Legacy PCA (Model 6300) やグレスビー9300™ (いずれも日本メディコ社製)，コーケン インフューザー™ R-INF TYPE PCA (高研社製) といった他社のPCAポンプにも同様の注意が必要である．サイホン効果による薬物の過剰投与については，シリンジポンプでの報告が存在し，シリンジポンプと患者間の高低差を少なくすること，静水圧で容易に溶液移動が生じないような抵抗の高いチューブを用いることが推奨されている[5,6]．

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Interactive CardioVascular and Thoracic Surgery

The short-term efficacy of fibrin glue combined with absorptive sheet material in visceral pleural defect repair

Masatoshi Gika, Masafumi Kawamura, Yotaro Izumi and Koichi Kobayashi

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Work in progress report - Experimental

The short-term efficacy of fibrin glue combined with absorptive sheet material in visceral pleural defect repair

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Abstract

Tissue sealants can prevent the occurrence of pulmonary air leakage, although few studies have evaluated the seal-breaking pressure properties of the various methods. We developed a new method for repairing visceral pleural defects which combines fibrin glue with a sheet material. We used an animal model to compare its efficacy with that of three current techniques up to 24 h after application. Under thoracotomy, 5×20 mm visceral pleural defects with a depth of 3 mm were made in beagles. The defects in the normal lungs were repaired using 1 of 4 methods: Method A, fibrin-glue double layer (fibrinogen solution was dripped, followed by thrombin solution); Method B, pack method (fibrin glue combined with polyglycolic acid sheet); Method C, rubbing and spray (fibrinogen was rubbed, followed by spraying of both fibrinogen and thrombin solutions); Method D, fibrin-glue-coated collagen fleece. The defects were repaired also in an emphysematous lung model using Method A, B or C. In the normal lungs, Method B showed significantly higher pressure resistance compared with the other methods at 5 min, 1 and 3 h post-application. Pressure resistance increased with time for all methods. In the emphysematous lungs, Method B showed significantly higher seal-breaking pressure than Methods A and C. Compared with existing tissue sealant methods, the pack method reliably controlled pulmonary air leakage immediately after application.

Keywords: Pulmonary air leakage; Visceral pleural defects; Tissue sealants; Emphysema; Fibrin glue

1. Introduction

Pulmonary air leakage is a common postoperative complication of respiratory surgery, and prolonged leakages can lead to longer hospitalization, and occasionally even thoracic infections [1]. The incidence of leakage increases particularly in procedures on the emphysematous lung. Depending on the location of the defect, and the degree of underlying emphysematous change if any, suturing or stapling can be extremely difficult. Furthermore, sutures may impede reinflation of the remaining lung. It is also technically difficult to suture pulmonary air leakages in the emphysematous lung during thoracoscopy.

The clinical benefits of tissue sealants in preventing pulmonary air leakages have been reported [2–8], but few studies have accurately assessed each method in terms of pressure resistance at the time of the repair and subsequent changes over time. We have previously reported differences in pressure resistance with different methods of fibrin glue application to repair pleural defects 5×10 mm in size with a depth of 2 mm. We found that the rubbing and spray method showed the highest sealing effect [9]. However, this method was not as effective when we increased the defect size to 5×20 mm with a depth of 3 mm. With these findings in mind, we developed a new method which combines the rubbing and spray method with an absorptive sheet to cover the pleural defect. In this study, we investigated the usefulness of this method up to 24 h after application. We also obtained preliminary data in the emphysematous lung.

2. Materials and methods

2.1. Animals

Female beagles, aged 5–7 months, weighing 8–10 kg (Toyota Trading Co., Kumamoto, Japan) were used.

2.2. Tissue sealants

The sealants were fibrin glue (Bolheal®, The Chemo-Sero-Therapeutic Research Institute, Kumamoto, Japan) and fibrin-glue-coated collagen fleece (TachoComb®, ZLB Behring Co., USA). The absorptive sheet was a non-woven sheet (Neoveil®, Gunze Ltd, Kyoto, Japan), 0.15 mm in thickness. The sheet is loose, and highly elastic.

2.3. Production of pleural defects in normal canine lung

Animals ($n=65$) were intubated under general anaesthesia (0.25 mg atropine sulfate s.c., 30 mg/kg pentabarbitol sodium i.v., 10 mg suxamethonium chloride i.v.) and placed

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on controlled ventilation. Right thoracotomy was done. With airway pressure maintained at 10 cmH_2O, a pleural defect 5×20 mm in size with a depth of 3 mm was made on the surface of each of the anterior, middle and posterior lobes using a scalpel. The 5×20 mm defect size was determined by placement of a thin flexible metal film of this size on the visceral pleura. Bleeding was cauterised if needed, but sites where haemostasis was difficult to achieve were not used.

2.4. Pleural defect repair methods

The pleural defects were repaired using one of four methods. Three pleural defects were made per animal. Out of the four repair methods compared in this study, three different methods were randomly chosen to repair the three defects in one animal. Method A, fibrin-glue double layer; 0.4 ml of liquid fibrinogen is dripped onto the defect, followed by 0.4 ml of liquid thrombin. Method B, pack method; 0.2 ml of liquid fibrinogen is rubbed in gently, then a PGA sheet is placed over the defect, and 0.2 ml of liquid thrombin is sprayed onto the sheet, followed by 0.2 ml of liquid fibrinogen and 0.2 ml liquid thrombin sprayed together. The PGA sheet is cut to approximately 7×22 mm, allowing for an overlap of approximately 2 mm around the defect. Method C, rubbing and spray method; 0.2 ml of liquid fibrinogen is rubbed in gently, followed by 0.2 ml liquid fibrinogen and 0.2 ml liquid thrombin sprayed together. Method D, fibrin-glue-coated collagen fleece; cut to the same size as the PGA sheet, placed on the defect, and pressure applied for 5 min using dry gauze. Except for 5 min measurements, the chest was closed, and the animals were allowed to survive. Ketoprofen (100 mg) was administered for analgesia, and ampicillin sodium (150 mg) as prophylaxis against infection, both intramuscularly.

2.5. Measurement of seal-breaking pressure in the normal lung

The pressure resistance of the repaired site was measured at 1, 3, 6 and 24 h post-application under thoracotomy (Table 1). Seal-breaking pressure, the minimum positive airway pressure that produced air leakage, was measured separately for each of the three repairs, while the bronchi of the other two lobes were clamped. The highest airway pressure applied was 60 cmH_2O, because air leaks could occur from the pulmonary hilum at pressures above 60 cmH_2O. Following measurements at each time point, animals were killed by intracardiac injection of 1000 mg pentobarbital sodium.

2.6. Histopathological examination

Separate animals ($n=16$) were used for the preparation of tissue specimens from normal lungs, because tissue sealants could become separated from the lung surface when seal-breaking pressures were measured. The right lungs were removed at each time point post-application (methods A, 12 sites, B, 12 sites, C, 12 sites, D, 12 sites). The lungs were fixed in 10% formalin, and the region containing each repaired pleural defect was resected, embedded in paraffin and sliced into 3-μm sections and stained with haematoxylin-eosin.

Table 1
Number of sites repaired with each tissue sealant

Tissue sealant	Time point				
	5 min	1 h	3 h	6 h	24 h
Double layer	7	11	4	10	5
Pack method	9	11	4	10	5
Rubbing + spray	7	7	6	7	7
Fibrin-glue-coated collagen fleece	7	12	7	10	5

2.7. Measurement of seal-breaking pressure in the emphysematous lung

Animals with the same specifications as for the normal lung experiments were anaesthetised and laid on their right side. Under bronchoscopy, a solution of 40 mg elastase (elastase type I, porcine pancreas-derived, Sigma Co., St Louis, MO, USA) diluted in 20 ml of saline, was sprayed into each segment of the right lung [10]. Animals were allowed to recover after this treatment.

Six weeks after this treatment, the emphysema model animals were anaesthetised for seal-breaking pressure measurements as in the normal lung. On thoracotomy, the right lung was hyperinflated, the visceral pleura was rugged, and small airspaces were visible through the pleura. In some animals, these changes were not homogeneous, but experiments were performed in locations where these changes were evident. Pleural defects were repaired randomly as in the normal lung using Method A, B or C. Seal-breaking pressure was measured at 5 min post-application. Separate animals could not be prepared to acquire tissue specimens only, because of the time required to produce the animal emphysema model. Therefore, following seal-breaking pressure measurements, the right lung was removed, fixed in 10% formalin, and the region containing each repaired pleural defect was resected and embedded in paraffin, sliced into 3-μm sections, and stained with haematoxylin-eosin.

2.8. Data analysis

All data are expressed as mean ± standard deviation. Statistical analyses were performed using the unpaired t-test (StatView, SAS Institute Inc., Cary, NC, USA), with $P<0.05$ considered a significant difference.

All animal studies were approved by the School of Medicine, Keio University Institutional Animal Care and Use Committee. All animals received humane care in accordance with the Japanese Government Animal Protection and Management Law.

3. Results

3.1. Seal-breaking pressure in the normal lung

At 5 min, 1 h and 3 h post-application, Method B showed significantly higher seal-breaking pressure than the other methods. No significant differences were seen between

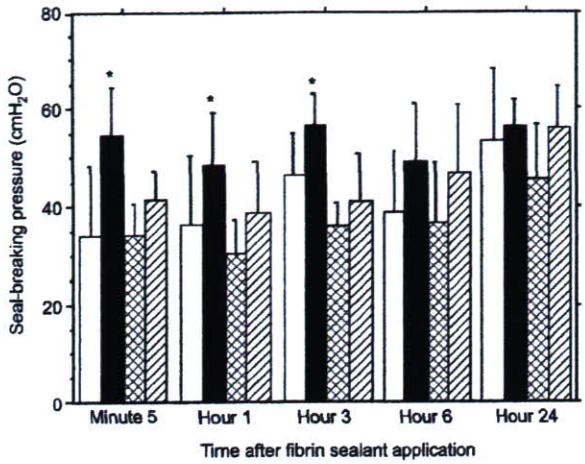

Fig. 1. Seal-breaking pressures at 5 min, and 1 and 3 h post-application, Method B showed significantly seal-breaking pressure resistance. No significant differences were seen between methods at 6 and 24 h. Method A: fibrin glue double layer [□]; Method B (pack method): fibrin glue + PGA sheet [■]; Method C: rubbing + spray [▨]; Method D: fibrin-glue-coated collagen fleece [▨].

methods at 6 and 24 h (Fig. 1). On direct observation under thoracotomy, each tested material was securely attached to the visceral pleura. Adhesion between the parietal pleura was not apparent.

3.2. Histopathological findings in repaired normal lungs

On histology, there was adequate attachment of the tissue sealant to the underlying lung surface with each repair method. There were no apparent traces of excess bleeding or inflammation in the adjacent lung, or pleura (Fig. 2).

3.3. Seal-breaking pressure in the emphysematous lung

On direct observation, each tested material was securely attached to the visceral pleura. At 5 min post-application in the emphysematous lungs, Method B showed significantly higher seal-breaking pressure than Method A or C (Method A vs. B vs. C; 25 ± 7 ($n=11$) vs. $37\pm12^{*}$ ($n=12$) vs. 25 ± 7 ($n=7$) cmH_2O, $^{*}P<0.05$). Seal-breaking pressure was lower in the emphysematous lung than in the normal lung for all repair methods (normal vs. emphysematous, cmH_2O, Method A: 34 ± 15 vs. 25 ± 7, $P=0.1$; Method B: 55 ± 10 vs. 37 ± 12, $P=0.01$; Method C: 34 ± 6 vs. 25 ± 7, $P=0.02$), and a significant difference was seen in Methods B and C. In a separate preliminary experiment, no significant difference was seen between normal and emphysematous lungs in the seal-breaking pressure at 5 min without repair (18 ± 3 vs. 16 ± 7 cmH_2O, $P=0.4$).

3.4. Histopathological findings in the emphysematous lung

Histological examination revealed emphysematous changes in the lung parenchyma, particularly in the proximity of the pleura. As seal-breaking pressures had already been measured in this group, partial separation of the tissue sealant had occurred, although overall, sufficient attachment of the tissue sealant to the underlying lung surface

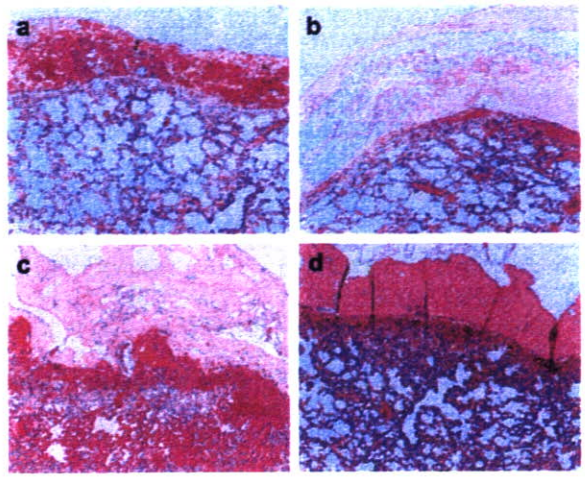

Fig. 2. Representative histopathological findings of normal lung at 24 h following repair. (a) Double layer; (b) pack method; (c) rubbing + spray; (d) fibrin-glue-coated collagen fleece (H&E, 4×).

was seen with each repair method. There were no apparent traces of excess bleeding or inflammation in the adjacent lung, or pleura (Fig. 3).

4. Discussion

Currently available fibrin glue products are derived from plasma, in most cases human, and hence carry similar risks as blood transfusion. Despite these potential drawbacks, the benefits of fibrin glue and fibrin-glue-coated collagen fleece in preventing recurrence of pulmonary air leakages has been reported [2–8,11,12]. However, none has included an experimental evaluation of pressure resistance and efficacy of each method.

Our pack method used a 0.15-mm-thick PGA sheet, which was soft and flexible because of the rough weave, and therefore presumably, fitted well into the irregular contour of the defects. Thrombin easily penetrates the sheet, but

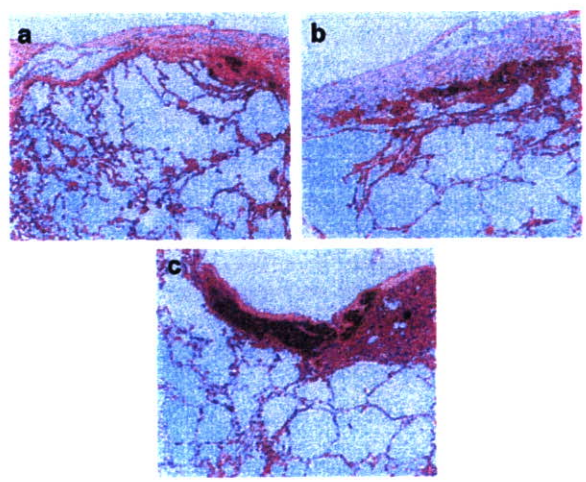

Fig. 3. Representative histopathological findings of emphysematous lung at 24 h following repair. (a) Double layer; (b) pack method; (c) rubbing + spray (H&E, 4×).

fibrinogen does not. By applying the PGA sheet over the fibrinogen, the solution is retained by the sheet fibres allowing for secure fibrin formation between the defect and the sheet following thrombin application. When solutions A and B are then sprayed together, there is an even layer of fibrin covering the defect, both under and over the sheet [13].

In the clinical setting of postoperative management, high airway pressures can occur with coughing at recovery from anaesthesia and at extubation, possibly causing rupture of the repair site and air leakage. Once a repaired pleural defect ruptures, it takes time to heal by endogenous fibrin and regeneration of the pleural membrane. The present study along with our previous study, have shown that pressure resistance increases with time, regardless of the repair method, and so it is of particular importance that there is high pressure resistance immediately post-application. In our study the only method to fulfil this expectation was the pack method (Fig. 1).

Direct observation showed that with the pack method, the sealants remained adherent to the defect, as well as to the adjacent pleura, with the lung either underinflated or hyperinflated. The fibrin-glue-coated collagen fleece separated from the lung at pressures exceeding 40 cmH_2O, causing air leakage. With the fibrin-glue double layer, the efficacy was inconsistent. The rubbing and spray method showed high pressure resistance with small pleural defects, but with larger defects we were unable to demonstrate significant superiority. We have not investigated whether this difference was due primarily to the increase in defect size or depth. To this end, further studies are necessary to determine the defect size or depth the pack method is able to withstand.

Histological examination of the normal lung post-application showed adequate coverage of the lung surface for all repair methods. No findings that might reflect differences in pressure resistance between application methods were found. This is thought to be because the pressure resistance of tissue sealants derives from their adhesive strength in relation to physical factors such as the elasticity of the pleural membrane and the airway pressure acting on the repair site, making it difficult to assess through histological examination. Although adverse findings were not apparent in any of the methods tested up to 24 h, long-term studies are needed to further assess efficacy, and safety.

On emphysematous lungs, control of air leakage becomes even more difficult. Suturing or stapling may cause new pleural defects in these cases. Furthermore, sutures may impede reinflation of the remaining lung, further reducing postoperative lung capacity in patients already suffering from impaired pulmonary function. We compared seal-breaking pressures at 5 min post-application, at which time point significantly higher seal-breaking pressure was achieved with the pack method than with the other methods. Fibrin-glue-coated collagen fleece was not evaluated due to lack of animal number, but we assume that it would not stay adherent to the hyperinflated emphysematous lung due to its stiffness. Seal-breaking pressures were lower with all methods in the emphysematous lung than in the normal lung. Little additional information regarding differences in pressure resistance between methods in the emphysematous lung was gained through histological examination. Further long-term studies are needed in this area.

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ORIGINAL ARTICLE

Phase II trial of gemcitabine and docetaxel in patients with completely resected stage IIA–IIIA non-small-cell lung cancer

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Abstract
Background Few clinical phase II studies using non-platinum doublet as adjuvant chemotherapy following complete resection of non-small-cell lung cancer (NSCLC) have been published, so this clinical study was designed to evaluate the toxicity profile and efficacy of the non-platinum doublet of docetaxel (DOC) + gemcitabine (GEM).
Methods Eligibility criteria included completely resected NSCLC, pathological stage II or IIIA, younger than 76 years old, and performance status 0–1. Treatment consisted of DOC 60 mg/m^2 on day 8, and GEM 1,000 mg/m^2 on days 1, 8, and 15 every 4 weeks (4 cycles). The GEM dosage was decreased to 800 mg/m^2 after the initial 21 patients because 3 patients developed interstitial lung disease (ILD).
Results Thirty-five patients (male/female 21/14) were enrolled. The median age was 62 years (range 47–74), with five (14.3%) over the age of 70. Performance status was 0 in 34 patients. The diagnosis was ad in 28 patients, sq in 6, and adsq in 1. The pathological stage was IIA in 5 patients, IIB in 1 and stage IIIA in 29 (82.9%). All patients underwent at least one cycle of chemotherapy, with 29 patients completing three cycles of chemotherapy and 23 (66%) had four cycles. The main grade 3/4 toxicities comprised neutropenia ($n = 21$, 60%), thrombocytopenia ($n = 3$, 8.6%), anorexia ($n = 4$, 11.4%), and ILD ($n = 3$, 8.6%), which responded well to corticosteroids. There were no treatment-related deaths. The 4-year recurrence-free survival rate was 42.9%, and the 4-year survival rate was 65.8%.
Conclusions The non-platinum doublet regimen of DOC + GEM as adjuvant chemotherapy following complete resection of NSCLC is feasible, with good compliance, the only problem being ILD.

Keywords Non-platinum doublet · Adjuvant chemotherapy · Non-small-cell lung cancer · Docetaxel · Gemcitabin · Surgery

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Introduction

The 5-year survival rates for patients with fully resected non small cell lung cancer (NSCLC) are around 40%, and no better than 40–60% even for patients with clinical stage IB/II disease, which cannot be considered as satisfactory results [1]. Postoperative chemotherapy is part of the standard therapeutic strategy for breast and colorectal cancers, and randomized clinical trials have been conducted to evaluate the efficacy of it for NSCLC. In the latter part of the 1970s chemotherapy based on cisplatin (CDDP) was shown to be effective in cases of advanced NSCLC, so a number of CDDP-based adjuvant regimens have been trialed and a meta-analysis published by the Non-small Cell Lung Cancer Collaborative Group in 1995 found

that these reduced mortality rates in comparison with surgery alone [2]. Although several large-scale clinical trials have subsequently been conducted and recently three large-scale randomized trials containing platinum-based chemotherapy have reported their positive results [including cisplatin-based chemotherapy (IALT) [3], CDDP ± NVB (JBR.10 [4]), ANITA trial [5])], at the time of commencing the present study the clinical benefits of postoperative adjuvant chemotherapy for patients with NSCLC had not yet been established.

On the other hand, in cases of inoperable NSCLC a doublet chemotherapy regimen including third-generation agent has been shown to significantly increase survival times in comparison with earlier doublet or triplet regimens [6–9]. Similar therapeutic effects have been reported for platinum (Pt)- and non-Pt-based doublet regimens in cases of inoperable NSCLC [10, 11].

Although we can anticipate improved survival rates with postoperative adjuvant chemotherapy regimens including third generation anticancer agents in comparison with surgery alone, we can also expect poor feasibility for a regimen of four courses of chemotherapy administered to postoperative patients. Considering that higher completion rates can be anticipated with non-Pt doublets, we planned a phase II trial of postoperative adjuvant chemotherapy for NSCLC using the new agents docetaxel (DOC) and gemcitabine (GEM).

Methods

We aimed for four courses of postoperative chemotherapy using DOC and GEM in patients with completely resected NSCLC, pathological stage II or IIIA. The primary endpoints were completion rates and adverse events, and the secondary endpoints were the recurrence-free survival rate and survival period.

Patient selection criteria were as follows: (1) pathologically complete resection; (2) histologically confirmed NSCLC; (3) age 75 years or less; (4) no double cancer (metachronous or synchronous); (5) postoperative pathological classification IIA, IIB or IIIA; (6) performance status (PS) of 0 or 1 according to the Eastern Cooperative Oncology Group (ECOG) criteria; (7) no pretreatment; (8) no postoperative infection or fever suggestive of infection; (9) appropriate blood test results (bone marrow function: WBC $\geq$ 3,000 per mm^3, Hb $\geq$ 10 g/dL, Plt $\geq$ 100,000 per mm^3; renal function: serum Cr $\leq$ 1.5 mg/dL, CCr $\geq$ 40 ml/min; hepatic function: serum AST, ALT $\leq$ 2 $\times$ upper limit normal); (10) no other conditions rendering patient medically unsuitable for treatment with anticancer agents (e.g. cardiac disease, severe diabetes); and (11) informed consent given to participate in this study.

Patients who filled all these criteria were enrolled in the study at between 2 and 4 weeks following surgery, and treatment was commenced before the sixth postoperative week.

Treatment protocol

On days 1, 8 and 15, GEM 1,000 mg/m^2 was administered as a 30-min intravenous (IV) infusion and DOC 60 mg/m^2 as a 1-h IV infusion on day 8. Chemotherapy cycles were repeated every 4 weeks, for a total of four courses. Both gemcitabine and docetaxel were administered by approved dosage and administration in Japan.

Patients received granulocyte-colony-stimulating factor (G-CSF) infusions after each cycle at the discretion of the investigator, but G-CSF was not used routinely.

Patients in whom treatment was interrupted due to grade 3/4 myelosuppression were re-treated with 75% of both the GEM and DOC dosages once the hematological parameters had returned to the levels prescribed in selection criterion 9.

Follow-up investigations

Every 3 months after surgery, patients attended the Outpatients Department for an periodic examination including plain chest radiography. Thoracic CT scanning, abdominal ultrasonography or CT scanning, and bone scintigraphy were examined once a year for at least 3 years following surgery.

Statistical analyses

Recurrence-free survival rates and cumulative survival periods were calculated using the method of Kaplan–Meier.

Results

Patient characteristics

A total of 35 patients (21 males, 14 females) were enrolled from the three institutions between August 2000 and 2002 (average age 62 years (range 47–74), five patients (14.3%) >70 years) (Table 1). The PS was zero in 34 patients, and one in 1. All patients were evaluated

Table 1 Patient characteristics

	All patients ($n = 35$)
Gender, no. (%)	
Male	21 (60%)
Female	14 (40%)
Age (years)	
Median	62
Range	47–74
ECOG PS	
0	34 (97%)
1	1 (3%)
Histology	
Adenocarcinoma	28 (80%)
Squamous cell carcinoma	6 (17%)
Adenosquamous cell carcinoma	1 (3%)
Surgical procedure	
Segmentectomy	1 (3%)
Lobectomy	32 (91%)
Bilobectomy	2 (6%)
Pathological stage	
Stage IIA	5 (14%)
Stage IIB	1 (3%)
Stage IIIA	29 (83%)

ECOG Eastern Cooperative Oncology Group, *PS* performance status

in terms of treatment toxicity and feasibility, and survival.

Treatment summary

The number of cycles of treatments administered to the patients is shown in Table 2. The first 21 patients were administered GEM 1000 mg/m^2, but after three patients developed interstitial lung disease (ILD), the dosage was decreased to 800 mg/m^2 for all subsequent patients. The average total GEM dosage was therefore 8736 mg/m^2, or 76.9% of the planned total dosage (calculations made on the basis of GEM 800 mg/m^2 on days 1, 8 and 15 as the full dosage for patients 22–35). The average total DOC dosage was 198.8 mg/m^2, or 82.9% of the planned total dosage.

Table 2 Treatment summary

	All patients ($n = 35$)
Cycle number	
1	35 (100%)
2	33 (94%)
3	29 (83%)
4	23 (66%)
Average no. of cycles	3.43
Mean total dose administered	
Gemcitabine	8736 mg/m^2 (76.9% planned)
Docetaxel	198.8 mg/m^2 (82.9% planned)

Causes of treatment suspension

The causes of treatment suspension, and the number of cycles completed when treatment was ceased, for the 12 patients who did not complete four courses are shown in Table 3. Cessation was due to adverse events in ten cases, the most common being grade 3/4 neutropenia, followed by pneumonitis, and there was one case each of cancer recurrence and patient refusal.

Adverse events

The main adverse events and their grades are shown in Table 4. Grade 3/4 neutropenia occurred in 21 patients, and grade 4 in two (5.7%). All subsequent chemotherapy was ceased in four patients with grade 3/4 neutropenia on the decision of the treating physician. Grade 3 thrombocytopenia occurred in three patients (8.6%), grade 3 anemia occurred in one patient (2.9%), 17 patients (48.6%) complained of nausea, but there was only one case of grade 3 nausea, 18 patients became anorexic (51.4%), four (11.4%) of whom required fluid supplementation (grade 3), ten patients (28.6%) recorded abnormal liver function tests but only one case was serious (2.9%), peripheral neuropathy occurred in six patients (17.1%), but no grade 3/4 cases, and although six patients (17.1%) complained of dyspnea, the diagnosis of ILD was made in three patients (8.6%) only, on the basis of the physical and radiological findings.

The three patients who developed ILD had all been administered GEM at the dosage of 1,000 mg/m^2, with the onset of symptoms occurring during the second course in one patient, and during the third course in two patients. The initial presentation was of dyspnea on exertion in one case, and fever in the other two cases. Subsequent thoracic CT scans revealed interstitial opacities in all three cases, and the diagnosis of drug-induced interstitial pneumonitis was made. The

Table 3 Causes of treatment suspension

	Cycle number at suspension		
	1	2	3
Grade 3 transaminitis	1	–	–
Grade 3 allergic reaction	1	–	–
Grade 3 pneumonitis	–	1	2
Grade 3 anorexia	–	–	1
Grade 3/4 neutropenia	–	–	3
Infection with grade 4 neutropenia	–	1	–
Recurrence	–	1	–
Refusal by patient	–	1	–

Table 4 Summary of adverse events

Adverse event	All patients[a] ($n = 35$)	Worst toxicity grade[b]				
		0	1	2	3	4
Hematological						
Neutropenia	33	–	2	10	19	2.
Thrombocytopenia	8	–	2	3	3	0.
Anemia	14	–	9	4	1	0.
Nonhematological						
Fever	7	–	3	4	0	0.
Nausea	17	–	11	5	1	0.
Anorexia	18	–	11	3	4	0.
Transaminitis	10	–	7	2	1	0.
Dyspnea	6	–	3	0	3	0.
Pneumonitis	3	–	0	0	3	0.
Peripheral neuropathy	6	–	3	3	0	0

[a] Each patient was counted once for each adverse event and assigned the worst toxicity grade reported for that patient during the study. Treatment related events were assessed as possibly, probably, or definitely related to treatment

[b] Common terminology criteria for adverse events, Version 3.0, were used to grade adverse events

highest recorded C-reactive protein (CRP) value for each of these patients was 1.9, 12.1 and 18.3 mg/dL, respectively. All three patients were given oxygen therapy and methyl-prednisolone (PSL) therapy (500 mg daily for 3 days, then PSL 20 mg daily gradually tapering the dosage for one patient, 80 mg and 50 mg, respectively, then gradually tapering for the other two patients). Symptoms resolved rapidly within 2–3 days of commencing PSL and the radiological signs also cleared relatively rapidly as the PSL dosage was reduced. None of the patients required assisted ventilation. Chemotherapy was ceased in all three patients at the onset of ILD. Following these developments, the GEM dosage was reduced to 800 mg/m^2 for the 22nd patient onwards. There were no chemotherapy-related deaths.

Survival

The median follow-up period was 4 years and 4 months. Using the Kaplan–Meier method, the 4-year recurrence-free survival rate was 42.9%, and the 4-year cumulative survival rate was 65.8% (Fig. 1).

Discussion

The results of the 2003 International Adjuvant Lung Cancer Trial Group (IALT) Phase III comparative trial with patients with completely resected NSCLC showed significantly prolonged survival times for Pt doublet chemotherapy versus surgery alone, including

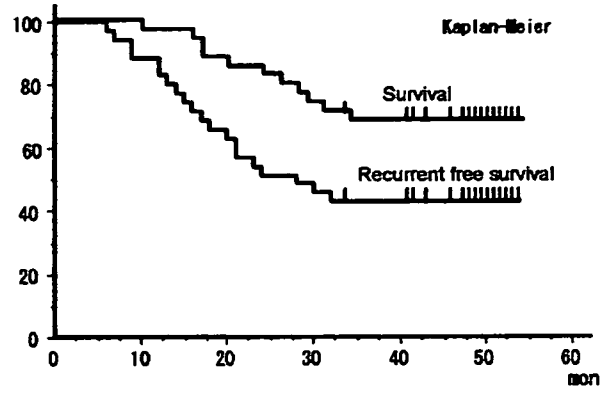

Fig. 1 Postoperative cumulative survival curve and postoperative recurrence-free survival curve (Kaplan–Meier method)

median survival time (MST) (50.8 vs. 44.4 months), recurrence-free survival (40.2 vs. 30.5 months), and 5-year survival rate (44.5 vs. 40.4%) [3, 12]. Winton et al. [4] reported that postoperative CDDP + vinorelbine (VNR) doublet chemotherapy significantly prolonged survival times. In comparison to surgery alone, MST (94 vs. 73 months), 5-year survival rate (69 vs. 54%) and recurrence-free survival rate (61 vs. 48%) were all significantly improved, and feasibility was also relatively favorable. In 2005, the Adjuvant Navelbine International Trialist Association (ANITA) published the final results of their trial of postoperative CDDP + VNR doublet chemotherapy. They reported that both the MST (36.3 vs. 20.7 months) and 5-year survival rate (51.2 vs. 42.6%) were more favorable for the adjuvant chemotherapy group than for the surgery alone group, and suggested that, since prolongation of the MST was particularly marked for stage II/IIIA disease, adjuvant chemotherapy should be considered standard treatment for these patients [5].

Subgroup analysis by stage confirmed that improvement in survival on stage II disease was significant in JBR.10 [4] and ANITA trial [5]. IALT [3] and ANITA trial showed a survival benefit from adjuvant chemotherapy in stage III disease.

Based on these reports, postoperative adjuvant chemotherapy is now becoming standard treatment for completely resected stage II and IIIA NSCLC, with Pt doublet regimens the most commonly used worldwide. However, few comparative trials have been conducted to determine the optimum regimen, and a consensus has not been reached as to what regimen should be used in which patients. We were unable to find any reports of phase II trials of postoperative adjuvant chemotherapy using non-platinum doublets.

Platinum doublet chemotherapy is also considered the standard treatment for advanced NSCLC, although

Georgoulias et al. [13] have reported a comparative trial of the non-Pt-based DOC + GEM regimen and the Pt-based DOC + CDDP regimen. No significant differences were seen between the two regimens in terms of efficacy, MST, or 1-year survival rates, but the lower toxicity for the DOC + GEM regimen suggested it might be more clinically advantageous. Subsequent phase III trials by Pujol et al. [10] and Georgoulias et al. [11] comparing VNR + CDDP and DOC + GEM doublet regimens similarly found no difference between regimens in survival, and superiority toxicity results for DOC + GEM. Matsui et al. [14] conducted a phase I/II study of the DOC + GEM doublet regimen, reporting an efficacy rate of 32.2%, similar to Pt-based regimens, but with less toxicity, suggesting that this regimen is a suitable candidate for future phase III trials. ASCO published the updated 2003 guidelines [15] for treatment of NSCLC. For stage IV NSCLC, non-platinum-containing chemotherapy regimens may be used as alternatives to platinum-based regimen in the first line. The results of those studies in cases of advanced NSCLC indicate that the DOC + GEM doublet regimen shows promise as postoperative adjuvant chemotherapy, and future phase III trials should elucidate its adverse reaction profile in comparison with Pt-based regimens.

In the present study, DOC + GEM doublet postoperative adjuvant chemotherapy was administered for a mean 3.43 courses (85.6%), with actual administered total dosages 76.9% of the planned dosage for GEM and 82.9% for DOC. Completion rates in JRB.10 [4] and ANITA trial [5] with the CDDP ± NVB regimen were 45 and 50%, respectively. Compliance with the protocol in ANITA was 89% for CDDP and 59% for NVB. These results indicate better feasibility than for the CDDP + NVB regimen which may be partially related to the low incidence of nausea and vomiting with DOC + GEM, making it suitable for administration on an outpatient basis.

The incidence of hematological toxicity in the present study was high, with grade 3/4 neutropenia detected in 60% of patients. The incidence of grade 3/4 neutropenia with DOC + GEM chemotherapy in patients with inoperable NSCLC has been reported as 10–19% [11, 16–18], so the present incidence with postoperative DOC + GEM is rather high. In comparison, the reported incidence of grade 3/4 neutropenia with postoperative CDDP + NVB chemotherapy was 73% in the Intergroup JBR.10 [4], and 84.6% in the ANITA report [5]. In two studies in which at least six courses of CDDP + NVB were administered to patients with inoperable NSCLC the reported incidence of grade 3/4 neutropenia was 57 [19] or 58% [20], indicating that this combination also produces a high incidence of grade 3/4 neutropenia postopeatively, similar to the DOC + GEM combination. There are as yet few reports of postoperative adjuvant chemotherapy using third generation anticancer agents, and it is unclear at present whether the present high incidence of grade 3/4 neutropenia is a general trend or not. Future studies with more patients, and possibly the prophylactic administration of G-CSF, might show increased feasibility and eliminate delays in treatment schedules.

Grade 3/4 thrombocytopenia was detected in three of the present patients (8.6%), which is a similar incidence to the 2–8% reported in studies [5, 13, 14, 16] of patients with inoperable NSCLC. All three of the present cases were grade 3 and none required platelet transfusions.

In terms of non-hematological toxicities, few of the present patients complained of severe nausea, and the DOC + GEM regimen could be administered on an outpatient basis. Grade 3 anorexia occurred in four patients (11.4%), although no record of grade 3 anorexia event could be found in reports of DOC + GEM chemotherapy in patients with inoperable NSCLC. Further investigation with more patients required to determine whether this is characteristic of postoperative chemotherapy.

The other important adverse event encountered in this study was interstitial pneumonitis (3 patients, 8.6%). With a reported incidence of the order of 5.2–23% [10, 16, 21, 22], pulmonary events can be considered characteristic complications of the DOC + GEM regimen. In a Japanese trial of DOC + GEM chemotherapy as second-line treatment in cases of advanced NSCLC, 12.3% of patients developed ILD, with three deaths, causing the premature closure of the study [21].

GEM is known to cause ILD, with incidences as high as 8% when it is used as monotherapy [23]. Pavlakis et al. [24] stated, "Unexpected peripheral edema and the noncardiogenic pulmonary edema may be explained by a capillary leak syndrome induced by gemcitabine. In view of structural and metabolic similarities between Ara-C and gemcitabine, perhaps the mechanism of lung injury is common to both these drugs". Referring to DOC, Read et al. [25] stated, "The lung biopsy suggests that docetaxel-induced pneumonitis might be a hypersensitive pneumonitis similar to that described for methotrexate". They added, "Docetaxel-induced pneumonitis is remarkable in its long duration. It is conceivable that docetaxel pneumonitis might result from taxane-induced alterations in leukocytes, with the resulting pulmonary insult lasting for the life span of the leukocyte". As all three of the present cases of ILD responded rapidly to

corticosteroid therapy, we suppose that their ILD might be induced by GEM. Patients undergoing postoperative adjuvant chemotherapy with this combination should be carefully monitored for the development of ILD, and factors contributing to the development of ILD need further eludication.

Conclusions

Although over 80% of the present patients had postoperative pathological stage IIIa NSCLC, favorable results were achieved with DOC + GEM postoperative adjuvant chemotherapy, with a 4 year recurrence-free survival rate of 42.9%, and a 4 year survival rate of 65.8%. Feasibility was relatively favorable for this regimen, despite being 'postoperative' in all cases, and high compliance can be anticipated for postoperative adjuvant chemotherapy. The incidences of neutropenia and anorexia were, however, higher than for the chemotherapy in patients with inoperable NSCLC.

In conclusion, this regimen warrants further study as one arm of postoperative adjuvant chemotherapy for NSCLC. Larger studies are needed to elucidate the adverse effect profile. The next step could be a randomized phase II study comparing DOC + GEM with another combination already used as postoperative chemotherapy for NSCLC, such as CDDP ± NVB.

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ORIGINAL ARTICLE

Clinical evaluation of chemosensitivity testing for patients with unresectable non-small cell lung cancer (NSCLC) using collagen gel droplet embedded culture drug sensitivity test (CD-DST)

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Abstract
Purpose In the present study, we prospectively evaluated the clinical feasibility and efficacy of collagen gel droplet embedded culture drug sensitivity test (CD-DST) in unresectable non-small cell lung cancer (NSCLC) without previous treatment.
Experimental design Eighty patients with unresectable NSCLC, aged less than 81 years old, PS 0–1, and with evaluable tumor lesions, entered the study. If the patient had CD-DST active drugs, more than three cycles of chemotherapy containing these drugs were administered. If the patient did not have CD-DST active drugs, the patient could choose any treatment including best supportive care.
Results Of the 80 patients in this study, CD-DST yielded results successfully in 49 patients (61.3%). CD-DST active drugs were present in 22 patients, and significantly more female patients had in vitro active anticancer agents than male ($P = 0.0008$). All of the patients with CD-DST active agents received chemotherapy including these agents. In these patients, the response rate was 72.7%, and median survival was 15.0 months. In the patients without CD-DST active agents, 11 patients received standard, empirical chemotherapy. In these patients, response rate was 0%, and median survival was 6.0 months.
Conclusions The results show that CD-DST is capable of selecting the responders and the respective optimal regimens, and also delineating the patients less likely benefit from treatment.

Keywords Chemosensitivity · Non-small cell lung cancer · Unresectable lung cancer · Chemotherapy · Collagen gel droplet embedded culture drug sensitivity test

Abbreviations

DOC	Docetaxel
PAC	Paclitaxel
CPT-11	Irinotecan
VNR	Veinorelbin
GEM	Gemcitabine
CDDP	Cisplatin
CBDCA	Carboplatin
VDS	Vindesine
VP-16	Etoposide
CD-DST	Collagen gel Droplet embedded culture Drug Sensitivity Test
HBSS	Hanks' balanced saline solution
FBS	Fetal bovine serum

Introduction

Over 70% of non-small cell lung cancer (NSCLC) patients have unresectable cancer by the time of diagnosis. Chemotherapy with so-called new generation chemotherapeutic agents as docetaxel (DOC), paclitaxel

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(PAC), irinotecan (CPT-11), veinorelbin (VNR), and gemcitabine (GEM) has achieved approximately 40% response rate with 4–5 months protraction of median survival by combination with platinum, compared with best supportive care [1]. However, there still remains 60% of non-responders. In view of these marginal response rates, many techniques to predict sensitive chemotherapeutic agents to a given patient have been pursued for various types of cancers. Though chemosensitivity test is one of these techniques, it has been scarcely applied to lung cancer because of the difficulty to obtain enough amounts of tumor cells for examination. To overcome this problem, we adopted collagen gel droplet embedded culture drug sensitivity test (CD-DST) [2] as an in vitro chemosensitivity assay.

In CD-DST procedures, extracted cancer cells are cultured three-dimensionally in collagen gel droplet. Three-dimensional culture with collagen matrix is preferable to establish cell culture from human cancer tissue [3]. This characteristic has made it possible to measure chemosensitivity with as little as 1×10^5 cancer cells, which would be present in one or two specimens biopsied by bronchoscope [4]. This system can also measure chemosensitivity with malignant effusion samples from pleural or pericardial effusions. Therefore, presumably CD-DST would be more feasible for in vitro drug sensitivity test for unresectable NSCLC.

To evaluate the clinical feasibility of CD-DST assay for unresectable NSCLC, we first assessed the success rates of CD-DST with various types of specimens. Next, to evaluate the validity of in vitro selection, we also assessed the correlation between in vitro sensitivity and clinical response, and survival of the patients treated with the regimens selected by CD-DST. In most of previous studies, the patients had received prior treatment [5]. The present prospective study was carried out in patients with previously untreated NSCLC.

Materials and methods

Protocol design

This prospective clinical study was approved by the appropriate institutional review boards. From 1998 to 2001, 80 patients with unresectable primary NSCLC, 80 years or younger, without previous treatment, with evaluable tumor lesions, with an Eastern Cooperative Oncology Group performance status of 0–1, and giving informed consent for participating in this study were eligible for examination of in vitro drug sensitivity with specimens from their tumors. Tumor specimens from metastatic cervical lymph nodes, intra-bronchial tumor, or malignant pleural effusion were biopsied or aspirated under local anesthesia after the patient's informed consent. Metastatic mediastinal lymph nodes were biopsied with mediastinoscope under general anesthesia for the purposes of staging and tissue procurement after the patient's informed consent.

In vitro data of CD-DST were available in 49 (61.3%) of 80 patients. Thirty-one patients' data of CD-DST were not available for various reasons which we described in the results. The 49 patients with CD-DST data, were eligible for the study to evaluate response to anticancer agents selected by CD-DST, and survival. Forty-nine patients were clinically staged according to UICC criteria adopted in 1997 [6].

Chemotherapy was selected on the basis of CD-DST results. When CD-DST showed two or more sensitive agents in a given patient, this patient was treated with the most active combination selected among popular regimens for NSCLC which contained the sensitive in vitro agents determined by CD-DST. If there were no generally accepted combination regimen including the sensitive in vitro drugs, standard two-drug-chemotherapy including the most sensitive agent was administered. When CD-DST selected only one chemotherapeutic agent, standard two-drug-chemotherapy including this agent was administered. In standard two-drug-chemotherapy including the one sensitive agent, the other drug was chosen by the clinician without any limitations. When CD-DST showed no sensitive agent, patients could choose any treatment including standard chemotherapy. Response was assessed after at least two courses of chemotherapy. Chemotherapy was continued up to six courses in patients who responded. Therapy was stopped at the time of progressive disease (PD). In case of tumor regrowth after chemotherapy or PD during chemotherapy, any other treatment could be chosen. All surviving cases were followed for more than 3 years.

Preparation of tumor cell suspensions

Each specimen except for malignant effusion were minced finely aseptically with a scalpel, suspended in Hanks' balanced saline solution (HBSS), and digested in a cell dispersion enzyme solution (10% EZ®, Nitta Gelatin Inc., Osaka, Japan) at 37°C for 1–3 h. The dispersed cancer cells were collected by centrifugation at 900 *g* for 3 min, filtered through an 80-μm nylon mesh, washed in HBSS, suspended in PCM-1® medium (Nitta Gelatin, Osaka, Japan), and incubated in a CG-flask® (collagen gel coated flask, Nitta Gelatin, Osaka, Japan) in a CO_2 incubator at 37°C for 24 h. The collagen gel in

the CG-flask was dissolved in the cell dispersion enzyme (10% EZ). This protocol allowed only viable cells, which could adhere to the collagen gel to be collected. It is checked with polarizing microscope whether the collected tumor cell suspension includes enough amount of tumor cells at this point.

Collagen gel droplet embedded culture

The prepared tumor cell suspension was added to a collagen solution (Collagen Gel Culture Kit Primaster®, Nitta Gelatin, Osaka, Japan) to produce a final cell density of 1×10^5 cells/ml. Three drops of collagen-cell mixture (30 μl/droplet) were placed in each well of a 6-well multiplate and allowed to form a gel at 37°C in a CO_2 incubator. Test for each anti-cancer agent was performed in triplicate. The final concentration was approximately 3×10^3 cells per collagen gel droplet. DF medium® (3 ml, Nissui Pharmaceutical Inc., Tokyo, Japan) containing 10% fetal bovine serum (FBS; Gibco, Gaitherberg, MD, USA) was overlaid on each well 1 h later, and samples were incubated in a CO_2 incubator at 37°C overnight.

In vitro chemosensitivity test

Nine anticancer drugs, Cisplatin (CDDP), carboplatin (CBDCA), vindesine (VDS), etoposide (VP-16), DOC, PAC, CPT-11, VNR, and GEM, were used for chemosensitivity analyses. These drugs were added at final concentrations adjusted to each clinical AUC and incubated for 24 h. The final concentration of each anti-cancer agent was as follows: CDDP 0.2 μg/ml, CBDCA 2.0 μg/ml, VDS 0.01 μg/ml, VP-16 1.0 μg/ml, DOC 0.1 μg/ml, PAC 1.0 μg/ml, CPT-11(SN38) 0.03 μg/ml, VNR 0.05 μg/ml, GEM 0.03 μg/ml. After removal of the medium containing the anticancer drugs, each well was rinsed with 4 ml HBSS each time, overlaid with 4 ml PCM-2 medium® (Serum Free Medium, Nitta Gelatin, Osaka, Japan), and incubated for 7 days. At the end of the incubation, neutral red was added to each well at a final concentration of 50 μg/ml, and incubated for 2 h. Each collagen droplet was fixed with 10% neutral formalin buffer, washed in water, air dried, and quantified by image analysis. The growth rates of control incubations were calculated as the total volume of living cancer cells on day 7 divided by the total volume of living cancer cells on day 1.

In vitro and in vivo correlation

The in vitro sensitivity was expressed as the percentage *T/C* ratio, where *T* was the total volume of living cancer cells of the treated group and *C* was the total volume of living cancer cells of the control group; a *T/C* ratio of 50% or less was regarded as being sensitive in vitro. Complete response (CR) was defined as the disappearance of all measurable lesions for at least 4 weeks. Partial response (PR) was defined as a decrease of 50% or more in the sum of the products of measurable lesions for at least 4 weeks without the development of new metastatic lesions. PD was defined as an increase of 25% or more in the sum of the products of measurable lesions or the appearance of new lesions. If no response or progression of the disease occurred during the chemotherapy, therapeutic effect was considered as no change (NC). The chemosensitivity result and the effect of chemotherapy was considered true positive if CR or PR was achieved after administration of two or more courses of chemotherapy including in vitro active drugs, and a true negative case was defined as NC or PD after administration of one or more courses of chemotherapy composed of in vitro non-active drugs.

Statistical analyses

A demographic data between groups were assessed by using χ^2 analysis or Mann–Whitney *U*-test. Survival was calculated from the date of protocol entry to the date of death or last known date alive. The Kaplan–Meier method was used to calculate the probability of survival as a function of time. A multivariate analysis was based on the Cox proportional hazards model. As the level of significance $P < 0.05$ was accepted.

This study was endorsed by Ethical Committee of Keio University Medical School. All patients proposed their informed consent by letter.

Results

Feasibility of in vitro analysis

Of 80 enrolled into Protocol Design, 49 patients (61.3%) had successful data acquisition of drug sensitivity testing. Feasibility rate of each specimen for CD-DST was 83.3% (10/12) in cervical lymph nodes, 71.4% (5/7) in mediastinal lymph nodes, 80.0% (20/25) in malignant pleural effusion, 31.3% (10/32) in intra-bronchial tumors, 100% (3/3) in other metastatic organs, and 100% (1/1) in pleural dissemination. The specimens from intra-bronchial tumors (31.3%) were less likely to have successful CD-DST, and specimens from resected tumors like cervical lymph nodes or metastatic organs were more likely to have successful CD-DST. Causes of unsuccessful

assays with intra-bronchial tumors were loss of viability of tumor cells, bacterial or fungal contamination, or insufficient number of tumor cells. Most of unsuccessful assays with malignant effusion were due to absence of tumor cells.

Patients characteristics

Characteristics of the 49 patients, whose in vitro data of CD-DST were available to evaluate the response to anticancer agents, are shown in Table 1. Stage IIIA patients were diagnosed as unresectable because of bulky metastases to mediastinal lymph nodes. Between two groups, with or without in vitro active drugs, there were no statistical differences in age, histological subtypes, T factors, N factors, M factors and clinical stages. Significantly more female patients had in vitro active anticancer agents than male patients ($P = 0.0008$).

Table 1 Characteristics of patients at entry, with or without CD-DST active drugs

	With CD-DST active drugs	Without CD-DST active drugs	χ^2-test
Age (years)			
65≥	8	9	$P = 0.537$
64<	14	18	
Median age	59	59	
Gender			
Male	11	25	$P = 0.0008$
Female	11	2	
Histological diagnosis			
Adenoca.	16	14	$P = 0.175$
Sq. ca.	4	4	
Large ca.	0	4	
Non-small ca.	2	5	
Clinical T factor			
T1	0	0	$P = 0.025$
T2	7	2	
T3	1	7	
T4	14	18	
Clinical N factor			
N0	0	0	$P = 0.984$
N1	1	1	
N2	10	12	
N3	11	14	
Clinical M factor			
M0	7	8	$P = 0.869$
M1	15	19	
Stage at initial diagnosis			
IIIA	0	2	$P = 0.357$
IIIB	7	6	
IV	15	19	
Initial treatment			
Chemotherapy	22	11	$P < 0.0001$
Radiotherapy	0	7	
B.S.C or unknown	0	9	

The frequency of in vitro sensitivity to each single chemotherapeutic agent by CD-DST

At our laboratory, CD-DST were examined in 782 primary NSCLC specimen. These include the 80 specimens enrolled in this study. More than half of these were primary tumors surgically resected. Among these, ranging from 401 to 575 drug sensitivity tests were examined successfully for each single agent. Table 2 shows the number of in vitro sensitive tumors ($T/C \leq 50\%$) to each single chemotherapeutic agent.

Chemotherapy selection

Doublet chemotherapy was administered to all of the 22 patients who had in vitro active anticancer agents. The combinations always included active in vitro agents. On the other hand, only 11 of 27 patients, who did not have in vitro active agents, were given standard empirical chemotherapy, as shown in Table 3. The average cycles of chemotherapy administered to each group were 3.68 and 2.44, respectively. There was a statistical difference between these groups ($P = 0.028$; Mann–Whitney U-test). Seven patients in the in vitro chemo-resistant group underwent chest radiotherapy only. Treatments for the remaining patients were best supportive care or unknown. In initial treatment selection, a significant difference was present between these two groups ($P < 0.0001$; χ^2-test).

Correlation between the results of in vitro testing and clinical response to chemotherapy

The patients with in vitro active agents were treated with 3.68 average cycles of chemotherapy based on

Table 2 The frequency of being sensitive in vitro to each single chemotherapeutic agent by CD-DST

Agent	No. of sensitive tumors (%)	Total
CDDP	133 (24.1%)	551
CBDCA	87 (19.6%)	443
VDS	129 (30.3%)	426
VP-16	100 (24.9%)	401
DOC	222 (38.6%)	575
PAC	133 (31.0%)	429
CPT-11	171 (33.6%)	509
VNB	136 (31.1%)	438
GEM	166 (39.6%)	419

A drug was considered CD-DST active when a *T/C* ratio was 50% or less

CDDP cisplatin, *CBDCA* carboplatin, *VDS* vindesine, *VP-16* etoposide, *DOC* docetaxel, *PAC* paclitaxel, *CPT-11* irinotecan, *VNR* veinorelbin, *GEM* gemcitabine

Table 3 Selection of drug combinations in this study, and the number of courses administered

	With CD-DST active drugs	Without CD-DST active drugs
CDDP + DOC	3	2
CDDP + CPT-11	0	2
CDDP + GEM	2	0
CDDP + VDS	3	1
CDDP + NVB	1	0
CDDP + ETP	0	2
CBDCA + PAC	1	1
CBDCA + GEM	1	0
CBDCA + DOC	2	0
CBDCA + CPT-11	2	0
CBDCA + DOX	0	1
DOC + NVB	2	0
PAC + NVB	1	0
DOC + GEM	4	0
UFT (Tegafur + Uracil)	0	2
Total	22	11
Average courses of chemotherapy (excluding two cases of UFT)	3.68 ± 1.56*	2.44 ± 1.42

*$P = 0.0282$ (Mann–Whitney U-test)

sensitivity results. These chemotherapies resulted in PR in 16 of 22 (72.7%), NC in 3 (13.6%), and PD in 3 (13.6%). The chemotherapy given to the patients without in vitro sensitive agents resulted in NC in 3 (27.3%) and PD in 8 (72.7%). True positive rate was 72.7% and true negative rate was 100%. Sensitivity and specificity were 100 and 64.7%, respectively. Accuracy was 81.8% (Table 4).

Drug sensitivity testing and survival

The median potential follow-up period of patients enrolled in this study was 3.5 years. Survival was measured from the beginning of examination of in vitro chemosensitivity. Median survival time (MST) of the patients treated with their in vitro optimal regimens was 15.0 months and that of the patients treated with empirical chemotherapy due to the lack of in vitro sensitive agents, was 4.5 months (Fig. 1). Multivariate analysis based on the Cox proportional hazards model showed that only the administration of in vitro optimal regimen ($P = 0.0272$) was significantly positive prognostic factor. M factor ($P = 0.0248$) was the significantly negative prognostic factor in this study (Table 5).

Table 4 Correlation of CD-DST activity with clinical response

	Clinical response			
With CD-DST active drugs	CR 0	PR 16	NC 3	PD 3
Without CD-DST active drugs	CR 0	PR 0	NC 3	PD 8

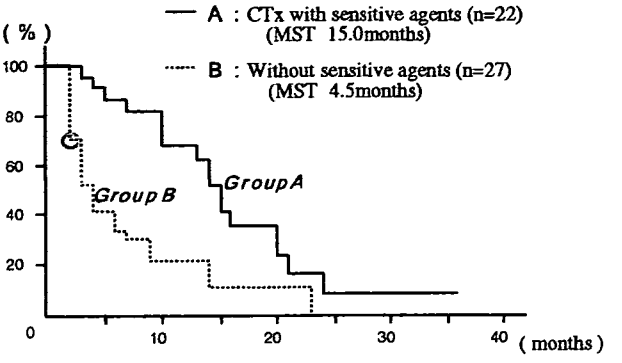

Fig. 1 Patient survival from the time of CD-DST. Two curves are depicted, each representing the survival of patients treated with in vitro selected sensitive ($n = 22$) and insensitive ($n = 27$) chemotherapeutic agents

Table 5 Multivariate analysis of potential factors affecting survival

Variables	χ^2	Risk ratio	95% confidence limits	
			Lower	Upper
Age	0.5572	1.010	0.977	1.044
Gender	0.9227	1.047	0.411	2.672
T factor	0.6499	0.897	0.561	1.435
N factor	0.1190	1.720	0.870	3.400
M factor	0.0248	2.447	1.120	5.344
Chemotherapy	0.3157	0.614	0.236	1.593
Administration of CD-DST active drugs	0.0272	0.286	0.094	0.869

Discussion

To obtain tumor cells for drug sensitivity testing from the patients with unresectable NSCLC, resection of metastatic superficial lymph nodes, mediastinoscopic biopsy for metastatic mediastinal lymph nodes, aspiration of malignant pleural or pericardial effusion, and bronchoscopic biopsy for intra-bronchial tumors were attempted. Though among these procedures only mediastinoscopy was performed under general anesthesia. Mediastinal lymph nodes were sampled also to evaluate pathological stage and operability. With any type of procedure, it was not so easy to procure enough amounts of viable tumor cells from the patients with unresectable lung cancer.

We adopted a new type of in vitro chemosensitivity testing, CD-DST. In this technique, extracted cancer cells are cultured three-dimensionally in collagen gel droplets. Three-dimensional culture with collagen matrix is most suitable for establishing cell culture from human cancer tissue or malignant effusion. This

characteristic makes it possible to measure chemosensitivity with as little as 1×10^5 cancer cells which are usually included in one or two specimens biopsied by bronchoscope [2–4, 7]. Among biopsy procedures for NSCLC, bronchoscopic biopsy seems to be least invasive to procure specimens for in vitro drug sensitivity assay. This study showed that the specimen obtained by bronchoscopic biopsy was available for in vitro drug sensitivity testing by use of CD-DST, though its feasibility was not satisfactory. In this study, the success rate of this assay with bronchoscopic biopsy specimens was the lowest among the procurement methods (31.3%). However, total feasibility in these patients was 61.3% and this value was better than previously reported data in other methods [8–10]. This suggests that CD-DST was feasible in various kinds of biopsy techniques, and is a preferable assay method for in vitro drug sensitivity test with these types of biopsy specimen from unresectable NSCLC.

Between the two groups, with or without in vitro sensitive drugs, significant differences were observed in gender ($P = 0.0008$) and clinical T factor ($P = 0.025$). Certainly, it was reported that female with NSCLS had higher response to Gefitinib as a molecular targeting agent, and UFT as adjuvant chemotherapeutic agent after surgery [11, 12]. However, no study has clearly described that females with NSCLC are more likely to show better response to empirical chemotherapy for NSCLS. Further examinations seem to be required to explain the deviation in gender. Patients without in vitro sensitive agents were more likely to have advanced T factors. We have data that the frequency of having in vitro sensitive agents by CD-DST tended to decrease as clinical stage advanced (unpublished data). However, as Table 1 showed no statistical differences between these two groups in clinical N factor and M factor, it is unlikely that only T factor influenced in vitro chemosensitivity. Also, the deviation in clinical T factor could not be explained with our small data.

In 49 patients with successful drug sensitivity testing, 22 (44.9%) had in vitro sensitive chemotherapeutic agents, and 16 (72.7%) of 22 treated with chemotherapy selected by CD-DST had PR. Though only 11 patients of remaining 27 without in vitro sensitive chemotherapeutic agent had chosen to be administered empirical chemotherapy, there was no responder among these 11 in vitro non-responders treated with empirical chemotherapy. These results imply that good correlation exists in this study between in vitro drug sensitivity and the patients' clinical response.

When we started this study, new chemotherapeutic agents as DOC, PAC, CPT-11, VNR, and GEM became clinically available in our country. Then drug sensitivity test with these new agents by CD-DST also became available. As it became possible to measure chemosensitivities to these new agents, many kinds of new combination chemotherapy became available. Indeed, in this study 11 different regimens were selected for 22 patients with in vitro sensitive agents by CD-DST. Other studies have also shown that many types of new regimens can be selected for the patients with NSCLC [5, 8] by in vitro chemosensitivity tests. These results indicate the heterogeneity of optimal regimens for the patients with NSCLC. Furthermore, our study showed a good correlation between in vitro drug sensitivity and the patients' clinical response, a true positive rate of 72.7%. The other reports also showed satisfactory values of true positive rate [10, 13]. Though our data was relatively low, among three patients with the tumors showing NC in size against chemotherapy with in vitro sensitive agents we had two long-term NC. Collectively, these data indicate that in vitro chemosensitivity tests could select effective combinational chemotherapy for respective cancers with satisfactory true positive rate.

Our study showed preferable MST in the patients treated with in vitro sensitivity based regimens, and poor survival in the patients treated with in vitro non-sensitive agents. It was not permitted by Ethical Committee of Keio University Hospital to randomize the patients with in vitro sensitive chemotherapeutic agents into two groups, treated with in vitro guided chemotherapy or fixed standard chemotherapy, because it was thought that the patients suffering from the procurement for drug sensitivity testing should have the right to know the result of CD-DST with their own specimen. Administration of fixed empirical chemotherapy to the patients without in vitro sensitive chemotherapeutic agents was also not permitted, resulting in free choice of treatment by patients themselves. Therefore, the comparison of the survival curves between the patients with in vitro selected sensitive and insensitive chemotherapeutic agents has no meaning. Based on these conditions, to evaluate the influence of various factors on survival of the patients enrolled in this study, multivariate analysis was performed. This analysis showed only administration of in vitro sensitivity guided chemotherapy was a significant factor to prolong the survival of the patients with NSCLC, and M factor was a significantly negative factor for their survival. Chemotherapy itself had no significant influence on survival of the patients enrolled in this study.

The number of the patients enrolled in this study is small, though these data suggest that in vitro guided combinational chemotherapy to the patients