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原　著

原発性肺腺癌特異的マーカーの免疫細胞化学的検討

——Surfactant apoprotein A, Napsin A, Thyroid transcription factor-1——

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Key words：Surfactant apoprotein A—— Napsin A—— Thyroid transcription factor-1—— Primary lung adenocarcinoma——Immunocytochemistry

目的：原発性肺腺癌の同定に有効な免疫組織化学的マーカーである Surfactant apoprotein A（SP-A），Napsin A，Thyroid transcription factor-1（TTF-1）を用い，細胞検体における有用性を検討した．

方法：原発性肺癌80例（腺癌67例，扁平上皮癌10例，その他3例），転移性肺腫瘍20例（大腸癌9例，腎癌4例，膀胱癌3例，肝臓癌2例，乳癌2例），胸膜悪性中皮腫2例，胃癌10例，乳癌10例の切除材料からの捺印標本をアルコール固定し，SP-A，Napsin A，TTF-1の各抗原に対する抗体を用いた免疫染色を行った．

成績：原発性肺腺癌において，SP-Aは68.7%，Napsin AおよびTTF-1は76.1%の陽性率であった．そのうち非粘液産生型腺癌に限れば，SP-Aは73.7%，Napsin AおよびTTF-1では82.5%とより高い陽性率であった．その他の原発性肺癌，転移性肺腫瘍，胸膜悪性中皮腫，乳癌，胃癌はすべて陰性であった．なおNapsin AとTTF-1陽性症例はすべて一致し，SP-A陽性全症例を含んでいた．

結論：Napsin AおよびTTF-1は細胞検体における原発性肺腺癌の同定に有用である．

I. はじめに

肺は原発性のみならず転移性腫瘍も多い臓器で，肺外悪性腫瘍の剖検例では20～54%に肺転移が認められるとの報告もあり[1)]，肺に腫瘍性病変をみた場合は，常に原発性肺癌であるか転移性肺腫瘍であるのかを鑑別しなければならない．原発性肺癌と転移性肺腫瘍との鑑別は腫瘍の病期診断のみならず，手術適応や化学療法の薬剤選択等の治療方針決定においてきわめて重要である．

細胞検体においては，細胞形態が原発臓器に類似した比較的特異性の高い像を呈していれば原発性か転移性かの推定は可能であるが，特徴的な細胞所見を欠く場合や細胞量が少ない場合には鑑別困難なことが多い．このような場合，より客観的な鑑別根拠を得るために免疫細胞化学的な検索が有効となる．現在までに，多数の免疫組織化学的検討が行われ，Cytokeratin 7と20の組み合わせによる種々の臓器原発癌の染色パターンの解析[2)]や臓器特異性の高い抗原として前立腺癌におけるProstate Specific Antigenの発現などが病理組織診断の補助として，原発臓器推定のために実用されている．

Immunocytochemical study of specific immunohistochemical markers for primary lung adenocarcinoma——Surfactant aporotein A, Napsin A, Thyroid transcription factor-1——

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原発性肺腺癌においては肺胞界面活性物質の一種であるSurfactant apoprotein A（以下SP-A）や肺胞上皮への分化やsurfactant aporotein A, B, C, secretory proteinの産生促進因子として知られている38kdの核内蛋白でNkx2 Familyのホメオドメインの転写因子であるThyroid transcription factor-1（以下TTF-1）は特異性の高い免疫組織化学的マーカーとして知られている[3]．また，近年肺腺癌の蛋白二次元電気泳動の結果から得られたaspartic proteinaseであるNapsin Aも原発性肺腺癌に特異性の高い免疫組織化学的マーカーであることが明らかとなってきた[4]．

これらのマーカーが細胞検体においても肺腺癌特異性を示すことがわかれば，鑑別診断の補助的手段としてきわめて有効である．そこで，今回われわれはSP-A, Napsin A, TTF-1についてアルコール固定を行った細胞検体での有用性を検討した．

II. 対象・方法

1. 対象

原発性肺癌80例：腺癌67例（非粘液産生型57例，粘液産生性細気管支肺胞上皮癌6例，印環細胞癌4例），扁平上皮癌10例，大細胞神経内分泌癌1例，カルチノイド1例，腺様嚢胞癌1例，転移性肺腫瘍20例（結腸・直腸癌9例，腎癌4例，膀胱癌3例，肝癌2例，乳癌2例），胸膜悪性中皮腫2例，胃癌10例，乳癌10例の切除材料からの捺印標本を用いた．おのおのの組織型はTable 1に示す．

2. 方法

腫瘍最大割面から捺印標本を作製し，パパニコロウ染色において腫瘍細胞の存在を確認後，カバーグラスを剥離し，免疫細胞化学的検討を行った．

腫瘍からの捺印標本を95%アルコールにて湿固定後，0.6%過酸化水素加メタノールにて30分間，内因性パーオキシダーゼのブロッキングを行い，TTF-1標本のみTarget retrieval solution（DakoCytomation）を用い，90℃，20分間の賦活化処理を行い，20分間室温にて冷却させた．一次抗体として抗SP-Aモノクロナール抗体（1000倍希釈，帝人），抗Napsin Aモノクロナール抗体（400倍希釈，IBL），抗TTF-1モノクロナール抗体（100倍希釈，Neomarkers）を用い，室温にて30分間反応させた後，Envision＋ポリマー試薬（DakoCytomation）を室温で30分間反応させ，DABで発色を行い，ギルのヘマトキシリンにて核染色を行った．

なお，捺印標本の一部には正常肺胞上皮のコンタミネーションも認められたが，形態学的に明らかな腫瘍細胞のみを判定し，TTF-1は核に，SP-A, Napsin Aは細胞質に反応している腫瘍細胞が10%以上あれば陽性と判定した．

Table 1 Immunocytochemical expression of SP-A, napsin A, and TTF-1

	Total	SP-A (%)	Napsin A (%)	TTF-1 (%)
Primary lung cancer				
Adenocarcinoma	67	46 (68.7)	51 (76.1)	51 (76.1)
Nonmucus-producing	57	42 (73.7)	47 (82.5)	47 (82.5)
Mucus-producing				
Mucinous bronchiolo-alveolar carcinoma	6	—	—	—
Signet-ring adenocarcinoma	4	4 (100)	4 (100)	4 (100)
Squamous cell carcinoma	10	—	—	—
Large cell neuroendocrine carcinoma	1	—	—	—
Carcinoid tumor	1	—	—	—
Adenoid cystic carcinoma	1	—	—	—
Metastatic lung cancer				
Colon and rectum (adenocarcinoma)	9	—	—	—
Kidney (renal cell carcinoma, clear cell)	4	—	—	—
Urinary bladder (transitional cell carcinoma)	3	—	—	—
Liver (hepatocellular carcinoma)	2	—	—	—
Breast (ductal carcinoma)	2	—	—	—
Primary neoplasms of other organs				
Mesothelioma of pleura	2	—	—	—
Breast cancer (ductal carcinoma)	10	—	—	—
Gastric cancer				
Poorly differentiated adenocarcinoma	5	—	—	—
Signet-ring cell carcinoma	5	—	—	—

III. 結　　果

原発性肺腺癌においては，SP-A の陽性率は 46/67 例 (68.7%)，Napsin A，TTF-1 の陽性率は，それぞれ 51/67 例 (76.1%) であった．後二者の陽性症例はすべて一致しており，この陽性症例群のなかに SP-A 陽性全症例が含まれていた．また，非粘液産生型腺癌に限った陽性率は SP-A で 42/57 例 (73.7%)，Napsin A，TTF-1 で 47/57 例 (82.5%) と高い陽性率であった (Photo. 1)．杯細胞類似の形態を示す粘液産生性細気管支肺胞上皮癌では，SP-A，Napsin A，TTF-1 とも 6 例すべて陰性であった (Photo. 2)．一方，印環細胞癌 4 例は三抗体ともすべて陽性であった (Photo. 3)．三者のそれぞれの染色態度は，TTF-1 の陽性局在は核で強くびまん性に染まっており，背景の過染はほとんどみられなかった．また SP-A，Napsin A の陽性局在は細胞質であったが，Napsin A は顆粒状に，SP-A はびまん性に染まっているものが主体であり，核内細胞質封入体には陽性所見は認められなかった．背景は，SP-A，Napsin A ともにマクロファージも陽性となり，SP-A において過染したものがやや多くみられた．

また，腺癌以外の原発性肺癌 13 例と転移性肺腫瘍 20 例，胸膜悪性中皮腫 2 例，乳癌 10 例，胃癌 10 例はこれら三抗原はすべて陰性であった (Table 1)．

IV. 考　　察

今回検討した 3 種類の抗原はすべて原発性肺腺癌に特異性が高く，特に非粘液産生型の亜型については陽性率も非常に高かったことから非粘液産生型の原発性肺腺癌の同定には有効であると考えられた．また，粘液産生型においても，今回検討した 3 種類の抗原は，杯細胞類似の粘液産生性細気管支肺胞上皮癌がすべて陰性であったのに対し，細胞形態的に原発性か転移性かの鑑別が必要になることがあるであろう印環細胞癌において肺原発性のものがすべて陽性で，かつ胃の印環細胞癌 5 例はすべて陰性であったことから，原発性肺腺癌の組織亜型の推定においても補助的指標の一つになりうると考えられた．

現在までに，原発性肺腺癌特異マーカーの免疫染色による陽性率の検討は，ホルマリン固定を行った組織材料においての報告は多数あるが，アルコール固定を行った細胞検体における報告は少ない．

Gomez-Fernandez ら[5] は原発性肺腺癌における TTF-1 の陽性率が胸水のセルブロックで 76% であったのに対し，アルコール固定の塗抹標本を用いると 54% に低下したと報告している．これに対して池田ら[6] はアルコール固定した捺印標本において 95.3%，Liu ら[7] は胸水のアルコール固定塗抹標本とセルブロック標本の比較で陽性率がともに 86% で，細胞検体においても有用なマーカーであると報告している．今回の検討で示されたように，これらの報告による陽性率の差は対象とした肺腺癌の組織亜型の違いによっている可能性もあるが，われわれの結果でも TTF-1 の陽性率は 76.1%，非粘液産生型については 82.5% と比較的高い陽性率を示し，細胞検体でも十分に有用であると考えられた．

SP-A，Napsin A の陽性率はホルマリン固定パラフィン切片での免疫組織染色において Hirano ら[8] はそれぞれ 69.8%，90.7% であり，Ueno ら[9] は 61.5%，84.6% であったと報告している．われわれの結果でも SP-A は 68.7%，Napsin A は 76.1%，非粘液産生型に限れば SP-A は 73.7%，Napsin A は 82.5% という比較的高い陽性率となり，これら 3 種類の抗原はアルコール固定を行った細胞検体においても免疫組織化学染色と同等の有用性があると考えられる．

ただし，非粘液産生型において 3 種類の抗原がすべて陰性であった 10 例のうち 8 例がいわゆる低分化型であり，Hirano ら[8] は免疫組織化学的検討において SP-A，Napsin A の陽性率が高分化型では，それぞれ 91.7%，100% であったのに対し，低分化型では，それぞれ 50.0%，66.7% であったと報告し，Stenhouse ら[10] も免疫組織化学的検討において原発性肺腺癌全体での TTF-1 の陽性率は 78% であったが，低分化型では 40% であったと報告しているように，低分化なものでは 3 種類の抗原の陽性率は低下するものと考えられる．

日常の細胞診業務においては，細胞検体の標本枚数や出現する細胞数に限りがあり，一般に多種類の抗体を用いた免疫染色が困難である．そのため，パネルとしての抗体の選択は重要であると考えられる．特異性については今回用いた 3 種類の抗体とも同様に高かったが，陽性率については Napsin A と TTF-1 が SP-A を上回っていた．しかも SP-A 陽性症例は，すべて Napsin A および TTF-1 が陽性であった．また，判定再現性を考えると，TTF-1 について Gomez-Fernandez ら[5] は大型の立体集塊において細胞膜に陽性様の所見を呈することがあるが，アーチファクトであり注意が必要であるとしている．一般に細胞検体においては背景の性状や集塊の重積性の程度などにより偽陽性を起こすことがあることは確かであり，今回の検討においても SP-A において比較的背景の過染等が多く，判定が困難なものがあった．一方，陽性部位が同じ細胞質である Napsin A は顆粒状に陽性所見を呈してくることから判定

は比較的容易であり，TTF-1については核に染まったもののみを陽性とすることにより背景に関係なく容易に判定が行えた．これらを踏まえると現段階ではTTF-1とNapsin Aのいずれかが原発性肺腺癌の免疫細胞化学的マーカーの第一選択となると考えられる．

日常業務においての利便性を考えると，TTF-1が抗原賦活化等の前処理を必要とするのに対し，Napsin Aは必要としないため比較的容易に行える点でより優れている．ただし，それぞれの陽性部位がNapsin Aは細胞質，TTF-1が核と異なるために，他の抗原との免疫二重染色等を行う場合にも必要に応じ使い分けることが可能であり，この両者の利用価値は高いと考える．

今回の検討において，細胞検体における原発性肺腺癌の同定にはNapsin AとTTF-1はいずれも有用なマーカーであると考えられた．

本論文の要旨は第43回日本臨床細胞学会秋期大会（東京）にて発表した．

Abstract

Objective : We evaluated the usefulness of currently available immunohistochemical markers specific for primary lung adenocarcinoma i.e., surfactant apoprotein A (SP-A), napsin A, and thyroid transcription factor-1 (TTF-1), in cytological materials.

Study Design : The immunocytochemical expression of SP-A, napsin A, and TTF-1 was studied in 122 imprint smears from surgically resected cases, i.e., 80 primary lung cancers, 20 metastatic lung cancers, 2 malignant pleural mesotheliomas, 10 gastric cancers, and 10 breast cancers.

Results : The positivity of SP-A, in all primary lung adenocarcinomas was 68.7%, of Napsin A 76.1%, and of TTF-1 76.1%. These rates increased to 73.7%, 82.5% and 82.5% when limited to primary lung adenocarcinomas without mucin production. In contrast, none of the metastatic lung cancers, malignant pleural mesotheliomas, gastric cancers, or breast cancers were stained with any of the 3 markers. Cases that were positive for napsin A and TTF-1 were entirely identical, and included all cases that were positive for SP-A.

Conclusion : Both napsin A and TTF-1 are very useful immunocytochemical markers for identifying primary lung adenocarcinomas.

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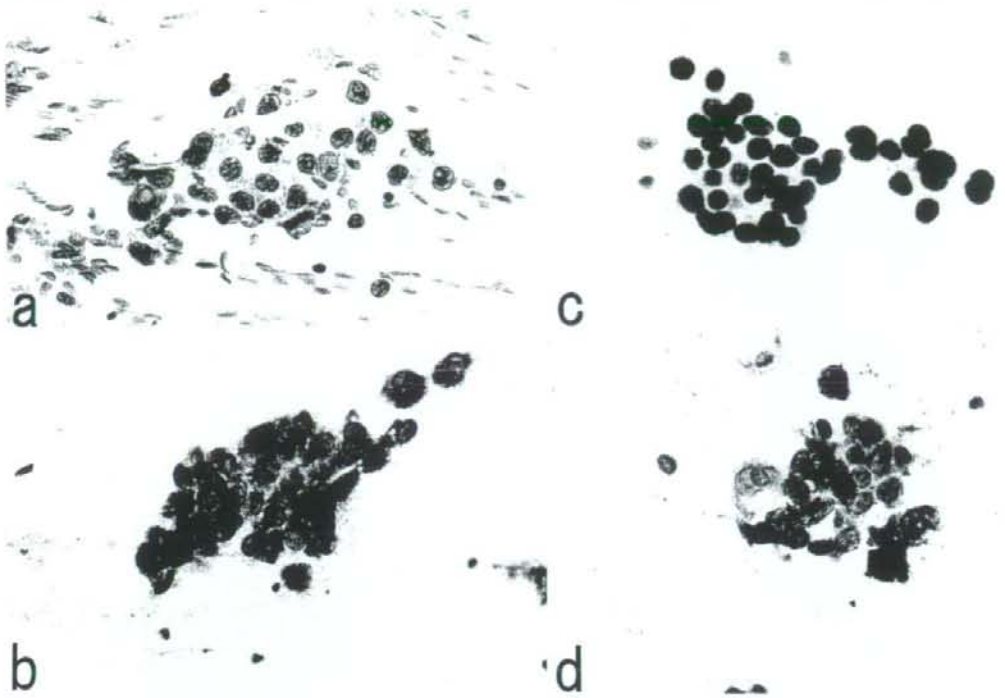

Photo. 1

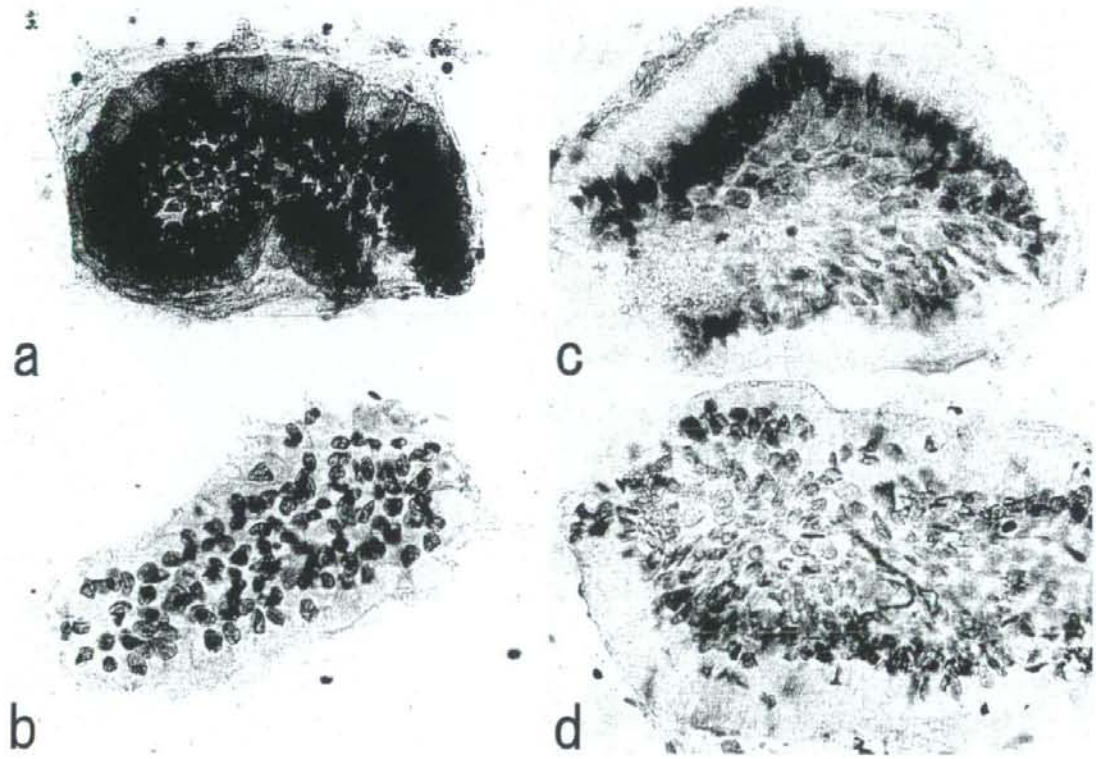

Photo. 2

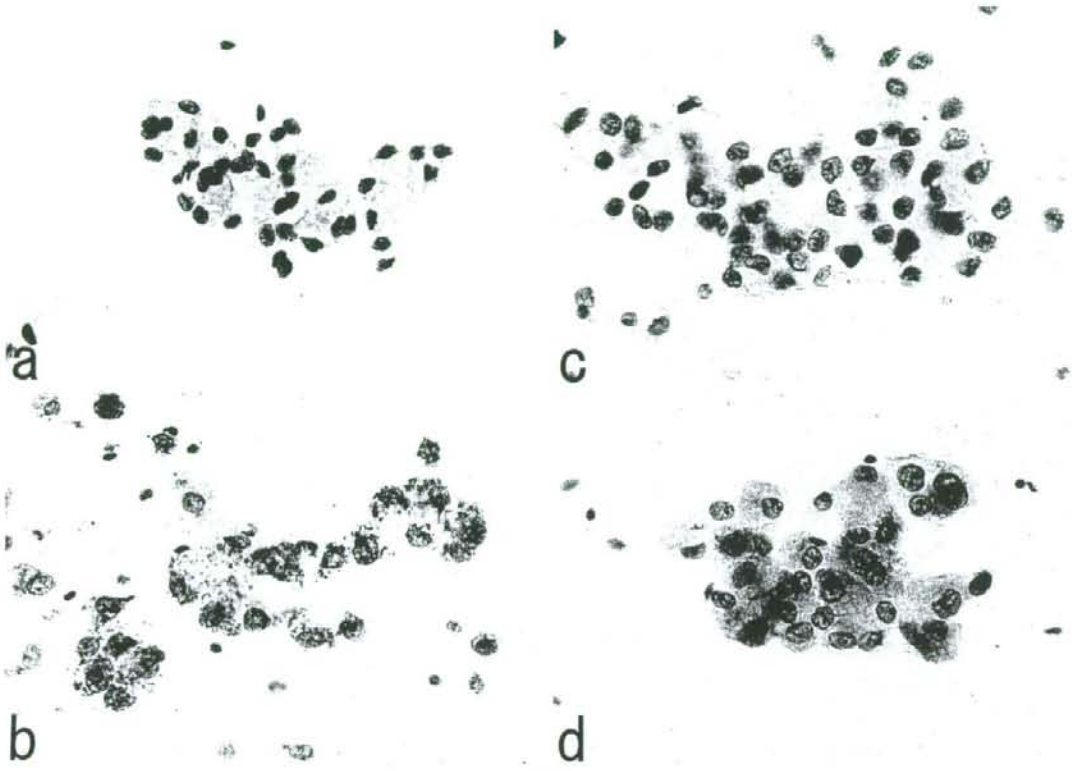

Photo. 3

写真説明

Photo. 1 Primary lung adenocarcinoma, nonmucinous type. a) Pap. stain×40. b), c), d) Immunocytochemical results showing strong reactivity of tumor cells to napsin A (b), TTF-1 (c), and SP-A (d). ×40.

Photo. 2 Primary lung adenocarcinoma, mucinous bronchioloalveolar carcinoma. a) Pap. stain×40. b), c), d) Immunocytochemical results showing no reactivity to napsin A (b), TTF-1 (c), or SP-A (d). ×40.

Photo. 3 Primary lung adenocarcinoma, signet-ring adenocarcinoma. a) Pap. stain×40. b), c), d) Immunocytochemical results showing strong reactivity of tumor cells to napsin A (b), TTF-1 (c), and SP-A (d). ×40.

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Short Communication

Randomised phase II trial of irinotecan plus cisplatin *vs* irinotecan, cisplatin plus etoposide repeated every 3 weeks in patients with extensive-disease small-cell lung cancer

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Patients with previously untreated extensive-disease small-cell lung cancer were treated with irinotecan 60 mg m^{-2} on days 1 and 8 and cisplatin 60 mg m^{-2} on day 1 with ($n=55$) or without ($n=54$) etoposide 50 mg m^{-2} on days 1–3 with granulocyte colony-stimulating factor support repeated every 3 weeks for four cycles. The triplet regimen was too toxic to be considered for further studies.

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Clinical Studies

Small-cell lung cancer (SCLC), which accounts for approximately 14% of all malignant pulmonary tumours, is an aggressive malignancy with a propensity for rapid growth and early widespread metastases (Jackman and Johnson, 2005). A combination of cisplatin and etoposide (PE) has been the standard treatment, with response rates ranging from 60 to 90% and median survival times (MSTs) from 8 to 11 months in patients with extensive disease (ED)-SCLC (Fukuoka *et al*, 1991; Roth *et al*, 1992). A combination of irinotecan and cisplatin (IP) showed a significant survival benefit over the PE regimen (MST: 12.8 *vs* 9.4 months, $P=0.002$) in a Japanese phase III trial for ED-SCLC (Noda *et al*, 2002), although another phase III trial comparing these regimens failed to show such a benefit (Hanna *et al*, 2006). Thus, irinotecan, cisplatin and etoposide are the current key agents in the treatment of SCLC. A phase II trial of the three agents, IPE combination, in patients with ED-SCLC showed a promising antitumour activity with a response rate of 77%, complete response (CR) rate of 17% and MST of 12.9 months (Sekine *et al*, 2003).

We have developed these IP and IPE regimens in a 4-week schedule where irinotecan was given on days 1, 8 and 15. The dose of irinotecan on day 15, however, was frequently omitted because of toxicity in both regimens (Noda *et al*, 2002; Sekine *et al*, 2003).

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The objectives of this study were to evaluate the toxicities and antitumour effects of IP and IPE regimens in the 3-week schedule in patients with ED-SCLC and to select the right arm for subsequent phase III trials.

PATIENTS AND METHODS

Patient selection

Patients were enrolled in this study if they met the following criteria: (1) a histological or cytological diagnosis of SCLC; (2) no prior treatment; (3) measurable disease; (4) ED, defined as having distant metastasis or contralateral hilar lymph node metastasis; (5) performance status of 0–2 on the Eastern Cooperative Oncology Group (ECOG) scale; (6) predicted life expectancy of 3 months or longer; (7) age between 20 and 70 years; (8) adequate organ function as documented by a white blood cell (WBC) count $\geqslant 4.0\times10^3\,\mu l^{-1}$, neutrophil count $\geqslant 2.0\times10^3\,\mu l^{-1}$, haemoglobin $\geqslant 9.5\,g\,dl^{-1}$, platelet count $\geqslant 100\times10^3\,\mu l^{-1}$, total serum bilirubin $\leqslant 1.5\,mg\,dl^{-1}$, hepatic transaminases $\leqslant 100\,IU\,l^{-1}$, serum creatinine $\leqslant 1.2\,mg\,dl^{-1}$, creatinine clearance $\geqslant 60\,ml\,min^{-1}$, and $PaO_2\geqslant 60$ torr; and (9) providing written informed consent.

Patients were not eligible for the study if they had any of the following: (1) uncontrollable pleural, pericardial effusion or ascites; (2) symptomatic brain metastasis; (3) active infection; (4) contraindications for the use of irinotecan, including diarrhoea, ileus, interstitial pneumonitis and lung fibrosis; (5) synchronous active malignancies; (6) serious concomitant medical

illness, including severe heart disease, uncontrollable diabetes mellitus or hypertension; or (7) pregnancy or breast feeding.

Treatment schedule

In the IP arm, cisplatin, 60 mg m^{-2}, was administered intravenously over 60 min on day 1 and irinotecan, 60 mg m^{-2}, was administered intravenously over 90 min on days 1 and 8. Prophylactic granulocyte colony-stimulating factor (G-CSF) was not administered in this arm. In the IPE arm, cisplatin and irinotecan were administered at the same dose and schedule as the IP arm. In addition, etoposide, 50 mg m^{-2}, was administered intravenously over 60 min on days 1–3. Filgrastim 50 μg m^{-2} or lenograstim 2 μg kg^{-1} was subcutaneously injected prophylactically from day 5 to the day when the WBC count exceeded 10.0×10^3 μl^{-1}. Hydration (2500 ml) and a $5HT_3$ antagonist were given on day 1, followed by an additional infusion if indicated in both arms. These treatments were repeated every 3 weeks for a total of four cycles.

Toxicity assessment, treatment modification and response evaluation

Toxicity was graded according to the NCI Common Toxicity Criteria version 2.0.

Doses of anticancer agents in the following cycles were modified according to toxicity in the same manner in both arms. Objective tumour response was evaluated according to the Response Evaluation Criteria in Solid Tumors (RECIST) (Therasse *et al*, 2000).

Study design, data management and statistical considerations

This study was designed as a multi-institutional, prospective randomised phase II trial. This study was registered on 6 September 2005 in the University hospital Medical Information Network (UMIN) Clinical Trials Registry in Japan (http://www.umin.ac.jp/ctr/index.htm), which is acceptable to the International Committee of Medical Journal Editors (ICMJE) (http://www.icmje.org/faq.pdf). The protocol and consent form were approved by the Institutional Review Board of each institution. Patient registration and randomisation were conducted at the Registration Center. No stratification for randomisation was performed in this study. The sample size was calculated according to the selection design for pilot studies based on survival (Liu *et al*, 1993). Assuming that (1) the survival curve was exponential for survivals; (2) the MST of the worse arm was 12 months and that of the better arm was 12 months × 1.4; (3) the correct selection probability was 90%; and (4) additional follow-up in years after the end of accrual was 1 year, the estimated required number of patients was 51 for each arm. Accordingly, 55 patients for each arm and their accrual period of 24 months were planned for this study.

The dose intensity of each drug was calculated for each patient using the following formula as previously described:

$$\text{The dose intensity (mg m}^{-2}\text{ week}^{-1}\text{)} = \frac{\text{Total milligrams of a drug in all cycles per body surface area}}{\text{Total days of therapy}/7}$$

where total days of therapy is the number of days from day 1 of cycle 1 to day 1 of the last cycle plus 21 days for both arms (Hryniuk and Goodyear, 1990).

Differences in the reason for termination of the treatment and the frequencies of grade 3–4 toxicities were assessed by χ tests. Survival was measured as the date of randomisation to the date of death from any cause or the date of the most recent follow-up for overall survival and to the date of disease progression or the date of death for progression-free survival (PFS). The survival of the arms was estimated by the Kaplan–Meier method and compared in an exploratory manner with log-rank tests (Armitage *et al*, 2002).

RESULTS

Patient characteristics

From March 2003 to May 2005, 55 patients were randomised to IP and 55 patients to IPE. One patient in the IP arm was excluded because the patient was ineligible and did not receive the study treatment. The remaining 109 patients were included in the analyses of toxicity, tumour response and patient survival. There were no differences between the two arms in any demographic characteristics listed (Table 1).

Treatment delivery

Treatment was well tolerated with respect to the number of cycles delivered in both arms (Table 2). Among reasons for termination of the treatment, disease progression was noted in nine (17%)

Table 1 Patient characteristics

	IP ($n=54$)	IPE ($n=55$)
Sex		
Female	11	8
Male	43	47
Age (years)		
Median (range)	63 (42–70)	62 (48–70)
PS		
0	11	12
1	42	41
2	1	2
Weight loss		
0–4%	38	43
5–9%	10	10
⩾10%	6	2

Table 2 Treatment delivery

	IP ($n=54$) No. (%)	IPE ($n=55$) No. (%)
Number of cycles delivered		
6[a]	—	1 (2)
4	41 (76)	36 (65)
3	6 (11)	6 (11)
2	3 (6)	6 (11)
1	4 (7)	6 (11)
Reasons for termination of the treatment[†]		
Completion	40 (74)	35 (64)
Disease progression	9 (17)	2 (4)
Toxicity	3 (6)	13 (24)
Patient refusal	2 (4)	4 (7)
Others	0 (0)	1 (2)
Total number of cycles delivered	192 (100)	186 (100)
Total number of omission on day 8	35 (18)	37 (17)
Total number of cycles with dose reduction	28 (15)	31 (17)

[†]$P=0.013$ by χ^2 test. [a]Protocol violation.

patients in the IP arm and in two (4%) patients in the IPE arm, whereas toxicity was noted in three (6%) patients in the IP arm and 13 (24%) patients in the IPE arm ($P=0.013$) (Table 2). The dose of irinotecan on day 8 was omitted in 35 (18%) cycles in the IP arm and 37 (17%) cycles in the IPE arm (Table 2). The total dose and dose intensity of cisplatin and etoposide were similar between the IP and IPE arms in the present study (Table 3).

Toxicity

The myelotoxicity was more severe in the IPE arm (Table 4). Grade 3 febrile neutropaenia was noted in 5 (9%) patients in the IP arm and 17 (31%) patients in the IPE arm ($P=0.005$). Packed red blood cells were transfused in 4 (7%) patients in the IP regimen and 14 (26%) patients in the IPE regimen ($P=0.011$). Platelet concentrates were needed in none in the IP regimen and 2 (4%) patients in the IPE regimen ($P=0.16$). Grade 3–4 diarrhoea was observed in 8 (15%) patients in the IP arm and 13 (24%) patients in the IPE arm ($P=0.262$). Grade 3–4 fatigue was more common in the IPE arm with marginal significance (2 *vs* 11%, $P=0.054$). The severity of other non-haematological toxicities did not differ significantly between the arms. No treatment-related death was observed in this study.

Table 3 Total dose and dose intensity

	3-week regimens in this study		4-week regimen*
	IP ($n=54$) Median (range)	IPE ($n=55$) Median (range)	IPE ($n=30$) Median (range)
Total dose ($mg\,m^{-2}$)			
Cisplatin	240 (60–240)	240 (60–360)	240 (60–240)
Irinotecan	420 (60–480)	390 (60–720)	563 (60–720)
Etoposide	0	600 (150–900)	600 (150–600)
Dose intensity ($mg\,m^{-2}\,week^{-1}$)			
Cisplatin	19 (14–25)	20 (16–34)	15 (12–15)
Irinotecan	33 (14–40)	35 (15–55)	35 (19–45)
Etoposide	0	48 (34–68)	37 (28–38)

*From our previous study (Sekine *et al*, 2003).

Table 4 Grade 3–4 toxicities

	IP ($n=54$)			IPE ($n=55$)		
	Grade 3	4	3+4 (%)	Grade 3	4	3+4 (%)
Leukocytopaenia	9	1	10 (19)	18	11	29 (53)*
Neutropaenia	17	11	28 (52)	24	28	52 (95)*
Anaemia	18	0	18 (25)	16	9	25 (45)
Thrombocytopaenia	2	0	2 (4)	13	0	13 (13)†
Febrile neutropaenia	5	0	5 (9)	17	0	7 (13)
Diarrhoea	8	0	8 (15)	11	2	13 (24)
Vomiting	4	0	4 (7)	3	0	3 (5)
Fatigue	1	0	1 (2)	5	1	6 (11)‡
Hyponatraemia	9	3	12 (22)	11	2	13 (24)
AST elevation	0	0	0 (0)	3	0	3 (5)
CRN elevation	1	0	1 (2)	0	0	0 (0)

*$P<0.001$; †$P<0.01$; and ‡$P=0.054$ by χ^2 test.

Response, treatment after recurrence and survival

Four CRs and 37 partial responses (PRs) were obtained in the IP arm, resulting in the overall response rate of 76 with 95% confidence interval (CI) of 65–87%, whereas six CRs and 42 PRs were obtained in the IPE arm, and the overall response rate was 87% with a 95% CI of 79–96% ($P=0.126$). Median PFS was 4.8 months (95% CI, 4.0–5.6) in the IP and 5.4 months (95% CI, 4.8–6.0) in the IPE arm ($P=0.049$) (Figure 1A). After recurrence, 22 (44%) patients in the IP arm and 8 (16%) patients in the IPE arm received etoposide-containing chemotherapy. The MST and 1-year survival rate were 12.4 months (95% CI, 9.7–15.1) and 54.8% (95% CI, 41.4–68.2%) in the IP and 13.7 months (95% CI, 11.9–15.5) and 61.5% (95% CI, 48.6–74.4%) in the IPE arm ($P=0.52$), respectively (Figure 1B).

DISCUSSION

This study showed that the IPE regimen in a 3-week schedule with CSF support produced a promising response rate, PFS and overall survival. Haematological toxicity in the IPE arm, however, was very severe in spite of the G-CSF support with the grade 3 febrile neutropaenia noted in 31% of patients.

In comparison between the 3-week IPE regimen in this study and the 4-week IPE regimen in the previous study, the delivery of cisplatin and etoposide was improved in the 3-week IPE regimen when compared with the 4-week IPE regimen at the cost of the irinotecan total dose. The response rate and MST were 87% and 13.7 months, respectively, in the 3-week IPE regimen and 77% and 12.9 months in the previous 4-week schedule, and toxicity profiles were comparable to each other (Sekine *et al*, 2003).

The MST of 12.4 months in the IP arm in this study was comparable to that of the previous phase III study, with an MST of 12.8 months (Noda *et al*, 2002). Thus, this study showed the reproducible excellent survival outcome of patients with ED-SCLC who were treated with the IP combination. In contrast, a recent American phase III study of the PE regimen *vs* IP regimen failed to show the superiority of the IP regimen to the PE regimen; the MST

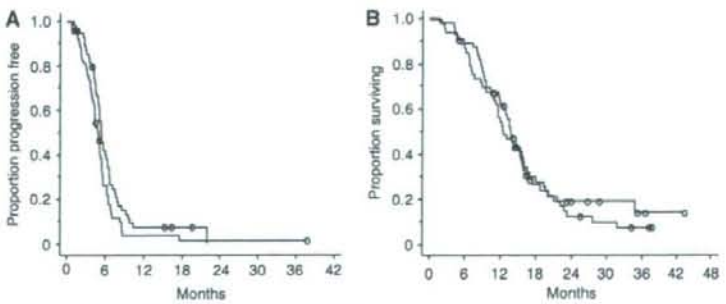

Figure 1 Progression-free survival (**A**) and overall survival (**B**). Thick line indicates the IPE regimen and thin line indicates the IP regimen.

for the PE regimen was 10.2 months and that for the IP regimen was 9.3 months (Hanna *et al*, 2006). The discrepancy between the Japanese and American trials may be explained by the different cisplatin dose schedules; cisplatin was delivered at a dose of 60 mg m^{-2} on day 1 every 3 or 4 weeks in the Japanese trials, whereas cisplatin was delivered at a dose of 30 mg m^{-2} on days 1 and 8 every 3 weeks in the American one. A platinum agent administered at divided doses was associated with poor survival in patients with ED-SCLC in our previous randomised phase II study (Sekine *et al*, 2003).

The issue of adding further agents to the standard doublet regimen has been investigated in patients with ED-SCLC. The addition of ifosfamide or cyclophosphamide and epirubicin to the cisplatin and etoposide combination produced a slight survival benefit, but at the expense of greater toxicity (Loehrer *et al*, 1995; Pujol *et al*, 2001). Phase III trials of cisplatin and etoposide with or without paclitaxel showed unacceptable toxicity with 6–13% toxic deaths in the paclitaxel-containing arm (Mavroudis *et al*, 2001; Niell *et al*, 2005). The results in these studies and the current study are consistent in the increased toxicity despite the G-CSF support and no definite survival benefit in the three or four drug combinations over the standard doublet in patients with ED-SCLC.

In conclusion, the IPE regimen was marginally more effective than the IP regimen, but was too toxic despite the administration of prophylactic G-CSF.

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Effect of Platinum Combined with Irinotecan or Paclitaxel against Large Cell Neuroendocrine Carcinoma of the Lung

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Background: The efficacy of chemotherapy in patients with large cell neuroendocrine carcinoma of the lung (LCNEC) remains unclear.
Methods: Of 42 consecutive patients with LCNEC, 22 with measurable disease receiving chemotherapy were enrolled as the subjects of this study. The clinical characteristics and objective responses to chemotherapy in these patients were analysed retrospectively.
Results: The distribution of the disease stage in the patients consisting of 21 males and one female (median age: 67 years; range: 47–78 years) was as follows: stage IIB ($n = 1$), stage IIIA ($n = 1$), stage IIIB ($n = 5$), stage IV ($n = 8$), and post-operative recurrence ($n = 7$). Chemotherapy consisted of cisplatin and irinotecan ($n = 9$), a platinum agent and paclitaxel ($n = 6$), paclitaxel alone ($n = 1$), cisplatin and vinorelbine ($n = 1$), cisplatin and docetaxel ($n = 1$), and a platinum and etoposide ($n = 4$). The objective response rate in the 22 patients was 59.1% (95% CI, 38.1–80.1). An objective response was obtained in five of the nine patients receiving irinotecan and five of the seven patients receiving paclitaxel. The progression-free survival, median overall survival and 1-year survival rates were 4.1 months (95% CI, 3.1–5.1), 10.3 months (95% CI, 5.8–14.8) and 43.0% (95% CI, 20.7–65.3), respectively. The median overall survival of the patients treated with irinotecan or paclitaxel was 10.3 months (95% CI, 0–21.8), and the 1-year survival rate of these patients was 47.6% (95% CI, 20.4–74.8).
Conclusion: Our results suggest that irinotecan and paclitaxel may be active against LCNEC.

Key words: lung cancer – large cell neuroendocrine carcinoma – chemotherapy – irinotecan – paclitaxel

INTRODUCTION

Neuroendocrine tumors of the lung can be placed in the biological spectrum ranging from typical to atypical carcinoid, which are tumors of low to intermediate grade malignancy, to large cell neuroendocrine carcinomas (LCNEC) and small-cell lung carcinomas (SCLC), which are high-grade malignant tumors. LCNEC was proposed as a separate category by Travis et al. in 1991, who recognized a type of poorly differentiated high-grade carcinoma exhibiting features of neuroendocrine appearance on light microscopy, immunohistochemistry, and/or electron microscopy (1). Several different terminologies and classifications have been proposed to date, and this class of tumors is likely to become widely recognized and included in the updated histological classification of the World Health Organization (2).

The clinical features of LCNEC have not yet been completely clarified. The prognosis of patients with surgically resected LCNEC is intermediate between that of an atypical carcinoid and SCLC, and is the same as that of resected non-small-cell lung carcinoma (NSCLC), except for stage I LCNEC, which has a poorer prognosis than that of stage I NSCLC (3–6). In a multi-institutional study in Japan, it was found that both LCNEC and SCLC were similarly aggressive and that there was no survival difference between the two types of lung cancer (7). In a small case series of LCNEC, we reviewed the records of patients with surgically resected,

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and patients treated medically who were autopsied before 1995, and determined that the chemosensitivity of LCNEC to cisplatin-based regimens may be intermediate between that of NSCLC and SCLC (8). Third generation cytotoxic agents developed in the 1990s, such as paclitaxel, docetaxel, gemcitabine, vinorelbine and irinotecan, have been shown to be active agents against advanced lung cancer, and combinations of platinum and one of the third generation cytotoxic agents have been shown to be superior in terms of prolonging the survival to the existing platinum-based combinations in both patients with NSCLC and those with SCLC (9–14). In the present study, we conducted a retrospective review of the records of our patients with LCNEC who had been treated with chemotherapy, and analysed the efficacy of the chemotherapy regimens.

PATIENTS AND METHODS

From April 1999 to January 2006, 42 patients were diagnosed as having LCNEC at our institution. Of these, one patient underwent surgery, four were treated with radiation therapy alone, and three received only supportive care. Of the 34 patients who had received chemotherapy, four who had also received concurrent radiotherapy and two without evaluable lesions were excluded from this study. In addition, six patients who entered a phase II trial of cisplatin and irinotecan combination for LCNEC were also excluded from this study, because their results will be published elsewhere. Thus, 22 patients were finally enrolled as the subjects of this study.

The histological confirmation of the diagnosis of LCNEC in the medically treated patients was based on examination of biopsy and/or cytology specimens. The histological or cytological diagnosis was reviewed by one of the authors (K.T.). We classified LCNEC according to the histopathological criteria proposed in the WHO classification. Immunohistochemical analysis was performed to confirm the neuroendocrine differentiation of the tumor cells (2).

Clinical information about the cases was obtained from medical records. All patients underwent a chest and abdominal computed tomography, a head computed tomography or magnetic resonance imaging and a bone scintigraphy in clinical disease staging before chemotherapy. The clinical disease staging was reassessed according to the latest International Union Against Cancer (UICC) staging criteria (15). The response to chemotherapy and the survival were assessed retrospectively. The objective tumor response was evaluated according to the Response Evaluation Criteria in Solid Tumor guidelines (16). The survival distributions for overall survival (OS) and progression-free survival (PFS) were estimated according to the Kaplan–Meier method (17). The OS was measured from the date of start of chemotherapy to the date of death or the last follow-up. For PFS, documented disease recurrence was scored as an event. All analyses were performed using the SPSS statistical software (SPSS version 11.0 for Windows; SPSS Inc, Chicago, IL).

RESULTS

The clinical characteristics of the 22 patients are summarized in Table 1. Surgical resected primary tumor, incisional biopsy of metastatic lesion, exploratatory thoracotomy, transbronchial or percutaneous biopsy and cytological examination were positive in seven, five, two, six and two patients, respectively. Thus, the histological diagnosis was made based on examination of a large tumor sample in 14 (63.6%) of the 22 patients. The marked predominance of men and smokers in this study was consistent with the demographic features of our previous LCNEC studies (6–8). One patient with stage IIB received chemotherapy and was enrolled to this study, because surgical resection and definitive radiotherapy were not indicated in this patient because of his poor pulmonary function. Abnormally high serum levels of CEA, NSE and proGRP at the start of chemotherapy were found in 52.4% (11/21), 72.7% (16/22) and 52.4% (11/21) of the patients, respectively.

Table 1. Patient characteristics

Characteristics		*n*	%
Gender	Male	21	95
	Female	1	5
Age	Median (range)	67 (47–78)	
Smoking history	Yes	21	95
	No	1	5
Performance status	0	7	32
	1	14	64
	2	1	5
Clinical stage	IIB	1	4
	IIIA	1	5
	IIIB	5	23
	IV	8	36
	Post-operative recurrence	7	32
Prior treatment	None	14	64
	Surgery	7	32
	Surgery for brain metastasis	1	5
	Radiotherapy	3	14
Site of metastasis	None	7	32
	Brain	2	9
	Lung	3	14
	Liver	5	23
	Bone	4	18
	Lymph node	6	27
	Others	3	14

The chemotherapy regimens used were as follows: cisplatin (80 mg/m^2, day 1) and irinotecan (60 mg/m^2, days 1 and 8) ($n = 6$); cisplatin (60 mg/m^2, day 1) and irinotecan (60 mg/m^2, days 1, 8 and 15) ($n = 3$); carboplatin (AUC = 6, day 1) and paclitaxel (200 mg/m^2, day 1) ($n = 5$); cisplatin (80 mg/m^2, day 1) and paclitaxel (175 mg/m^2, day 1) ($n = 1$); paclitaxel alone (80 mg/m^2, weekly) ($n = 1$); cisplatin (80 mg/m^2, day 1) and vinorelbine (20 mg/m^2, days 1, 8 and 15) ($n = 1$); cisplatin (25 mg/m^2, days 1, 8 and 15) and docetaxel (20 mg/m^2, days 1, 8 and 15) ($n = 1$); carboplatin (AUC = 5, day 1) and etoposide (100 mg/m^2, days 1–3) ($n = 3$); cisplatin (80 mg/m^2, day 1) and etoposide (100 mg/m^2, days 1–3) ($n = 1$). The median number of chemotherapy cycles was three (range, 1–5). One complete response and 12 partial responses were noted in the 22 patients, yielding an overall response rate of 59.1% (95% CI, 38.1–80.1) (Table 2). An objective response was obtained in five of the nine patients (55.6%) receiving irinotecan and five of the seven patients (71.4%) receiving paclitaxel. The toxicities related to these treatments were, in general, acceptable. Two patients received gefitinib after failure of the first-line chemotherapy, but none of them achieved an objective response. The overall PFS, median OS and 1-year survival rate of all the patients were 4.1 months (95% CI, 3.1–5.1), 10.3 months (95% CI, 5.8–14.8) and 43.3% (95% CI, 21.0–65.6), respectively (Fig. 1). The median OS of the patients treated with irinotecan or paclitaxel was 10.3 months (95% CI, 0–21.8), and the 1-year survival rate of these patients was 47.6% (95% CI, 20.4–74.8).

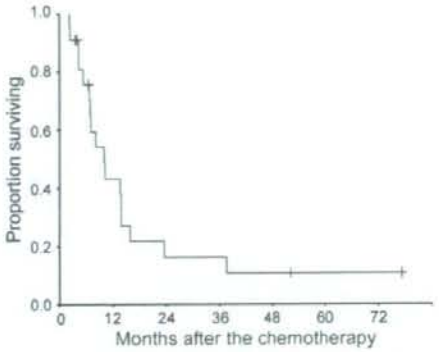

Figure 1. Kaplan–Meier curve for overall survival ($n = 22$). The median survival time was 10.3 months, and the 1- and 2-year survival rates were 43.3 and 16.2%, respectively.

DISCUSSION

In this study, the histological diagnosis of LCNEC was based on examination of a large tumor sample in 14 (63.6%) of the 22 patients, based on biopsies or cytological specimens in the remaining patients (36.4%). Numerous studies have demonstrated that the diagnosis of LCNEC is possible from biopsies or cytological specimens if a sufficient number of tumor cells can be obtained (8,18–21). To establish the pathological diagnosis of LCNEC in this series, we performed a pathological review of the biopsy and cytology specimens, because it was difficult to obtain large specimens of the tumor in these patients with advanced cancer treated medically.

We previously reported a response rate of 64% in 14 chemo-naïve patients with LCNEC who received cisplatin plus mitomycin, vindesine, or etoposide (8). In that study, however, patients with a diagnosis of poorly differentiated adenocarcinoma, poorly differentiated squamous cell carcinoma, large cell carcinoma and small cell carcinoma were selected, and then a diagnosis of LCNEC was made retrospectively by reviewing autopsy or surgically resected specimens. Thus, they were not consecutive, but highly selected patients. This explains, at least partly, the high response rate in the previous study. On the other hand, in the current study we analysed consecutive patients with a diagnosis of LCNEC that is established before treatment.

Rossi et al. showed that objective responses were observed in six (50%) of 12 patients with metastatic LCNEC who received a platinum and etoposide regimen, while no response was obtained in 15 patients receiving regimens for NSCLC treatment (cisplatin and gemcitabine in 10 patients, gemcitabine alone in two patients, and carboplatin and paclitaxel in three patients) (22). In addition, the patients receiving the platinum and etoposide regimen had a significantly better survival than the patients who received the other regimens (median survival time, 51 months versus 21 months). These survival data, however, sound too good for lung cancer patients with a metastatic disease. Neither patient characteristics nor explanation for

Table 2. Chemotherapy regimens and responses

Regimens		No. of patients	CR/PR/SD/PD	Response rate (%)
CPT-11-based	CDDP + CPT-11	9	0 / 5 / 3 / 1	55.6
PTX-based	CBDCA + PTX	5	0 / 3 / 2 / 0	60.0
	CDDP + PTX	1	1 / 0 / 0 / 0	–
	PTX	1	0 / 1 / 0 / 0	–
VNR-based	CDDP + VNR	1	0 / 1 / 0 / 0	–
DTX-based	CDDP + DTX	1	0 / 1 / 0 / 0	–
ETP-based	CBDCA + ETP	3	0 / 0 / 3 / 0	0
	CDDP + ETP	1	0 / 1 / 0 / 0	–
Total		22		59.1

CPT-11, irinotecan; PTX, paclitaxel; VNR, vinorelbine; DTX, docetaxel; ETP, etoposide; CDDP, cisplatin; CBDCA, carboplatin; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

such a long survival was presented in this report (22). Another case series of LCNEC showed that three patients with a stage IV disease received platinum-based chemotherapy (cisplatin and etoposide, carboplatin and gemcitabine, and cisplatin, docetaxel and gemcitabine) but none of them achieved an objective response. Of five patients who received gefitinib as salvage therapy, one achieved a partial response (23).

In this study, the clinical response rates of LCNEC to chemotherapy regimens containing irinotecan or paclitaxel were as high as 70%. The published response rates of NSCLC and SCLC to these regimens are 30–33% and 68–84%, respectively (10–14). The PFS of 4.1 months and median OS of 10.3 months were comparable to the results of previous randomized phase III trials that have reported PFS values of 4.1–6.9 months and median OS values of 9.3–12.8 months in extensive-stage disease SCLC (14). Thus, the response rate and survival of LCNEC were comparable with those of SCLC. Although our retrospective review of clinical data revealed heterogeneous approaches in treatment regimens, our results suggested that irinotecan and paclitaxel may be active agents against LCNEC. LCNEC exhibit both features of NSCLC and SCLC in terms of the morphology and immnohistochemistry, and these anti-cancer agents are effective against both of these types of lung cancer. Considered together, the combinations of cisplatin and irinotecan, and carboplatin and paclitaxel may be promising regimens for LCNEC.

To evaluate the efficacy of irinotecan- or paclitaxel-based combined chemotherapy for LCNEC, it is necessary to perform prospective phase II trials. However, such trials for LCNEC may be difficult to perform for the following reasons. First, patient accrual is problematic because LCNEC is a relatively rare tumor and accounts for only about 3% of lung cancer patients treated by surgical resection (6). It took us 7 years to accumulate 22 patients with LCNEC treated with chemotherapy. Besides, some studies have revealed the efficacy of adjuvant chemotherapy for both SCLC and NSCLC (24–26). Thus, when patients treated with platinum-based adjuvant chemotherapy regimens are excluded, few subjects with LCNEC with the diagnosis confirmed based on examination of large tumor specimens may remain. Therefore, these trials may only be possible as multi-institutional studies. Second, because it can sometimes be difficult to define the histology of LCNEC without examination of specimens large enough to appreciate the histological architecture and obtain reproducibility, pathological review by experts panel would be needed in these trials.

In conclusion, our results showed that irinotecan- or paclitaxel-based regimens may be as active against LCNEC as that against SCLC. A phase II multi-institutional trial is under way in Japan to elucidate the efficacy of cisplatin- and irinotecan-based therapy regimens against LCNEC.

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Conflict of interest statement

None declared.

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Serum Total Bilirubin as a Predictive Factor for Severe Neutropenia in Lung Cancer Patients Treated with Cisplatin and Irinotecan

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Objective: To clarify the association between pre-treatment total bilirubin (PTB) level and severe toxicity in patients receiving cisplatin and irinotecan.
Methods: We analyzed retrospectively the relationships of grade 4 neutropenia or grade 3–4 diarrhea and clinical variables including PTB and pre-treatment neutrophil counts (PNC) using a logistic regression model.
Results: One hundred and twenty-seven patients (93 men, 34 women; median age: 61 years; range: 24–74 years) received cisplatin (60 or 80 mg/m^2) on day 1 and irinotecan (60 mg/m^2) on days 1 and 8 every 3 weeks or on days 1, 8 and 15 every 4 weeks. Grade 4 neutropenia occurred in 29 patients (23%) and grade 3–4 diarrhea occurred in 13 patients (10%). Grade 4 neutropenia was associated with a higher PTB level (odds ratio: 4.9; 95% confidence interval: 1.4–17.7), a higher cisplatin dose (2.8, 1.0–7.8) and a lower PNC (1.5, 1.0–2.3). Grade 3–4 diarrhea was associated with liver metastasis (11.2, 2.2–57.4), a higher cisplatin dose (5.0, 1.2–21.3) and a lower PNC (2.0, 1.1–3.6).
Conclusions: PTB level was associated with the severity of neutropenia caused by cisplatin and irinotecan.

Key words: irinotecan – toxicity – lung cancer

INTRODUCTION

Although irinotecan is an active agent against several solid tumors, it sometimes exhibits serious adverse effects, the most common being bone marrow toxicity, in particular leucopenia and neutropenia, and ileocolitis, which leads to diarrhea (1–4). The severity of these toxicities varies greatly between individuals, and thus identifying pre-treatment factors that predict an increased risk for severe toxicities is a critical issue in the treatment of cancer patients undergoing chemotherapy.

Irinotecan needs to be activated by systemic carboxylesterases to SN-38 to exert its anti-tumor activity, which is mediated by the inhibition of topoisomerase I (5). Glucuronidation of SN-38 (SN-38G) by UDP-glucuronosyltransferase (UGT) 1A1 during biliary excretion is the primary route of detoxification and elimination. A higher ratio of plasma SN-38 to SN38-G has been correlated with severe diarrhea, suggesting that the efficiency of SN-38 glucuronidation is an important determinant of toxicity (6–8).

Genetic polymorphisms of the UGT 1A1 gene, such as the number of TA repeats in the TATA box that are associated with reduced transcriptional efficiency and functional activity, have been reported previously (7). Some studies have demonstrated an association between UGT1A1 polymorphisms and the risk for severe toxicity from irinotecan (6, 8–11).

The UGT1A1 enzyme is also responsible for hepatic bilirubin glucuronidation. Serum bilirubin levels, therefore, may reflect UGT1A1 activity and may also be associated with irinotecan activity and toxicity. The pre-treatment serum total bilirubin (PTB) level has been shown to be related to

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severe neutropenia in patients receiving 350 mg/m^2 of irinotecan (8). We extended this observation in patients receiving cisplatin and irinotecan to clarify the association between PTB and severe toxicity, including neutropenia and diarrhea, in these patients.

PATIENTS AND METHODS

Treatment Schedule

The subjects consisted of consecutive lung cancer patients who had received cisplatin and irinotecan therapy at the National Cancer Centre Hospital between February 1999 and May 2004. Irinotecan, diluted in 500 ml of normal saline, was given intravenously over 90 min at a dose of 60 mg/m^2 on days 1 and 8 or on days 1, 8 and 15. Cisplatin was given intravenously over 60 min after the irinotecan infusion at a dose of 60 or 80 mg/m^2 on day 1 with at least 2500 ml of hydration. The first phase I trial of irinotecan and cisplatin showed that 80 mg/m^2 of cisplatin on day 1 and 60 mg/m^2 of irinotecan on days 1, 8, and 15 were the recommended dose for phase II trials (12), and this dose schedule was used for subsequent phase II and phase III trials of non-small cell lung cancer (NSCLC) (13,4,14). The second phase I trial of this combination showed that 60 mg/m^2 of cisplatin on day 1 and 80 mg/m^2 of irinotecan on days 1, 8, and 15 were the recommended dose (15). A phase II trial for small cell lung cancer, however, showed that this dose schedule was too toxic, and thereafter the dose of irinotecan was reduced from 80 to 60 mg/m^2 (16). From the above, we used 80 mg/m^2 of cisplatin and 60 mg/m^2 of irinotecan for patients with NSCLC, and 60 mg/m^2 of cisplatin and 60 mg/m^2 of irinotecan for the other patients. Administration of irinotecan was omitted if any of the following toxicities were noted on days 8 and 15: a white blood cell count $<2.0 \times 10^9$/l, a platelet count $<75 \times 10^9$/l, or grade 1–3 diarrhea. Each course was repeated every 3 or 4 weeks until the occurrence of unacceptable toxicity, disease progression, patient's refusal to continue treatment, or the investigator's medical decision to stop treatment. To control for cisplatin-induced emesis, a 5-HT3 receptor antagonist and dexamethasone were given prior to cisplatin administration.

Study Design

We retrospectively reviewed the patients' clinical records, including patient characteristics (age, sex, Eastern Cooperative Oncology Group performance status, histology of primary disease, clinical stage, prior treatment, evidence of liver metastasis), the dose and schedule of chemotherapy, and pre-treatment complete blood counts and serum chemistry profiles. We defined 'severe toxicity' as grade 4 neutropenia or grade 3–4 diarrhea during the first cycle of chemotherapy, in accordance with the NCI-CTC Version 2.0 criteria. All patients were treated as in-patients, and complete blood counts and serum chemistry profiles were assessed at least once a week. PTB was defined as the serum total bilirubin level at fasting just prior to the administration of cisplatin and irinotecan.

Table 1. Patient characteristics

		No. of patients
Sex	Male/female	93/34
Age	Median (range)	61 (24–74)
Performance status	0/1/2	34/91/2
Histology	Non-small cell lung cancer	57
	Small cell lung cancer	63
	Others	7
Liver metastasis	Yes/no	18/109
Prior chemotherapy	Yes/no	17/110
PTB (mg/m^2)	Median (range)	0.6 (0.2–2.4)
PNC ($\times 10^9$/l)	Median (range)	4.1 (1.8–8.5)
Chemotherapy	CDDP (60) day 1 + CPT-11 (60) days 1.8 q3w	32
Regimens (mg/dl)	CDDP (60) day 1 + CPT-11 (60) days 1.8.15 q4w	39
	CDDP (80) day 1 + CPT-11 (60) days 1.8 q3w	24
	CDDP(80) day1 + CPT-11 (60) days 1.8.15 q4w	32

PTB, pre-treatment total bilirubin; PNC, pre-treatment neutrophil count.

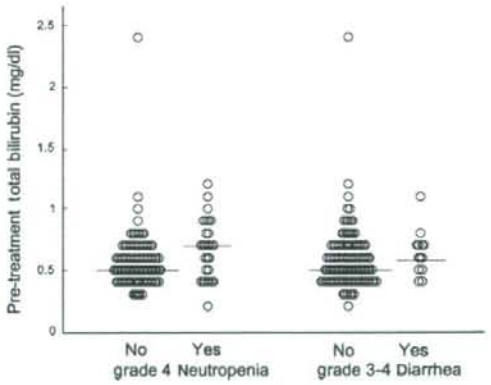

Figure 1. Association of PTB in patients who developed severe toxicity and in those who did not. The median PTB in patients who developed grade 4 neutropenia and those who did not was 0.7 (range, 0.2–1.2) mg/dl and 0.5 (range, 0.3–2.4) mg/dl, respectively ($P = 0.03$, Mann–Whitney U test). The median PTB in patients who developed grade 3–4 diarrhea and those who did not was 0.6 and 0.5 mg/dl, respectively ($P = 0.22$). The bars represent the median values.

Table 2. Univariate analysis of association between grade 4 neutropenia and pre-treatment clinical variables

	Neutropenia grade		Odds ratio (95% CI)
	Grade <4 ($n = 98$)	Grade 4 ($n = 29$)	
Sex			
Male	70	23	1
Female	28	6	0.65 (0.24–1.77)
Age			
Median (range)	61 (24–74)	65 (38–73)	1.04 (0.99–1.09)
Performance status			
0	29	5	1
1, 2	69	24	2.02 (0.70–5.80)
Liver metastasis			
No	82	27	1
Yes	16	2	0.38 (0.08–1.76)
Prior chemotherapy			
No	84	26	1
Yes	14	3	0.69 (0.19–2.60)
Treatment schedule			
Every 3 weeks	41	15	1
Every 4 weeks	57	14	0.67 (0.29–1.54)
Cisplatin dose (mg/m^2)			
60	56	15	1
80	42	14	1.24 (0.54–2.86)
AST (IU/l)			
Median (range)	22 (11–161)	22 (11–56)	0.98 (0.95–1.01)
ALT (IU/l)			
Median (range)	18 (6–266)	20 (5–67)	0.99 (0.97–1.02)
PNC ($\times 10^9$/l)			
Median (range)	4.4(2.0–8.5)	3.9 (1.8–8.3)	0.84 (0.61–1.14)
PTB (mg/dl)			
Median (range)	0.5 (0.3–2.4)	0.7 (0.2–1.2)	3.74 (0.70–19.9)
≤0.7	87	20	1
>0.7	11	9	3.56 (1.30–9.73)

AST, aspartate aminotransferase; ALT, alanine aminotransferase; PNC, pre-treatment neutrophil count; PTB, pre-treatment total bilirubin.

Statistical Methods

The Mann–Whitney U test was used to compare the PTB levels of patients who developed severe toxicity and those who did not. Possible explanatory factors were compared using a logistic regression model. A PTB threshold of ≤0.7 mg/dl was selected to categorize this variable because a total bilirubin level higher than 0.7 mg/dl has been correlated with a mutated UGT1A1 genotype and the occurrence of grade 4 neutropenia (8). Furthermore, sex, performance status, liver metastasis, prior chemotherapy, treatment schedule and cisplatin dose were defined as categorized variables, and age, AST, ALT and pre-treatment neutrophil count (PNC) were examined as continuous variables. Variables that seemed to be associated with severe toxicity ($P < 0.1$) were considered for inclusion in a multivariate analysis using a backward stepwise regression model. We performed these analyses using the SPSS statistical package (SPSS version 11.0 for Windows; SPSS Inc., Chicago, IL, USA).

RESULTS

A total of 127 consecutive patients with thoracic malignancy received cisplatin and irinotecan therapy. The patient characteristics are listed in Table 1. In all, two patients (1.5%) had

Table 3. Backward stepwise regression analysis of association between severe toxicity and pre-treatment clinical variables

Variable	Co-efficient	*P*	Odds ratio (95% CI)
Grade 4 neutropenia			
Cisplatin dose	1.04	0.04	2.84 (1.03–7.81)
PNC	0.42	0.04	1.53 (1.02–2.27)
PTB	1.59	0.02	4.93 (1.37–17.7)
Grade 3–4 diarrhea			
Liver metastasis	2.41	0.004	11.2 (2.18–57.4)
Cisplatin dose	1.61	0.03	5.00 (1.18–21.3)
PNC	0.67	0.03	1.96 (1.07–3.60)

Adjusted for age and PS.
PNC, pre-treatment neutrophil count; PTB, pre-treatment total bilirubin.

stage IIA disease, seven patients (5.5%) had stage IIIA disease, 26 patients (20%) had stage IIIB disease and 85 patients (67%) had stage IV disease. The median PTB level was 0.6 (range, 0.2–2.4) mg/dl and the median PNC was 4.1 (range 1.8–8.5) $\times 10^9$/l. A total of 93 patients (73%) received the planned doses without skipping the irinotecan administrations on day 8 or 15. Among the remaining 34 patients, the irinotecan on day 8 or 15 was omitted in 27 of 164 (16.5%) planned doses in patients with PTB level $\leq$0.7 mg/dl, while in 11 of 34 (32.4%) planned doses in patients with PTB level >0.7 mg/dl ($P = 0.053$). Thus, the actual irinotecan dose delivered was lower with marginal significance in patients with PTB level >0.7 mg/dl. Grade 4 neutropenia occurred in 29 (23%) patients and grade 3–4 diarrhea occurred in 13 (10%) patients.

The median PTB level was higher in patients who developed grade 4 neutropenia than in those who did not (0.7 and 0.5 mg/dl, respectively; $P = 0.03$) (Fig. 1), but PTB was not correlated with the presence or absence of grade 3–4 diarrhea ($P = 0.22$).

In a univariate analysis, grade 4 neutropenia was associated with only the PTB level ($\leq$0.7 versus >0.7 mg/dl; $P = 0.01$, Table 2). When PTB level was analyzed as a continuous variable, the association was not significant (OR: 3.74; 95% CI: 0.70–19.9; $P = 0.12$). In a multivariate analysis, grade 4 neutropenia was associated with the PTB level ($\leq$0.7 versus >0.7 mg/dl; $P = 0.02$), the cisplatin dose ($P = 0.04$), and PNC ($P = 0.04$, Table 3). In a univariate analysis, grade 3–4 diarrhea was associated with only liver metastasis ($P = 0.01$, Table 4). We analyzed serum levels of PTB and pre-treatment AST and ALT between patients with ($n = 18$) or without ($n = 109$) liver metastasis. The median (range) PTB was 0.6 (0.4–2.4) mg/dl in patients with liver metastasis and 0.6 (0.2–1.2) mg/dl in patients without liver metastasis ($p = 0.19$). In contrast, the median (range) levels of pre-treatment AST and ALT were 30 (16–114) IU/l and 30 (11–84) IU/l, respectively, in patients with liver metastasis and 21 (11–161) IU/l and 17 (5–266) IU/l, respectively, in patients without liver metastasis ($P = 0.0054$). In a multivariate analysis, grade 3–4 diarrhea was associated with liver metastasis ($P = 0.004$), the cisplatin dose ($P = 0.03$) and PNC ($P = 0.03$, Table 4).

DISCUSSION

This study showed that the PTB level was significantly associated with severity of neutropenia in patients treated with cisplatin and weekly irinotecan. Although irinotecan-induced toxicity can be reduced by skipping irinotecan on day 8, 15, or both, this dose modification is not enough to eliminate severe toxicity completely. In this study irinotecan was more frequently omitted on days 8 and 15 in patients with PTB level >0.7 mg/dl, and therefore, the association between PTB and irinotecan-induced toxicity may be underestimated. Thus, the PTB level, a simple routine measure in clinical practice, can be a useful predictive marker for irinotecan-induced toxicity.

The most compelling evidence for a genetic marker of toxicity caused by irinotecan therapy is seen with the *UGT* gene. In some retrospective pharmacogenetic studies, patients with at least one *UGT*1A1*28 allele encountered severe irinotecan-induced toxicity, compared with those with the wild-type genotype who were homozygous for the 6 TA repeat allele (6,9,10). In a prospective study, the *UGT*1A1 genotype was strongly associated with severe neutropenia in patients treated with irinotecan (8). More than 30 polymorphic variations have been reported to date for the *UGT*1A1 gene (17). Novel polymorphisms (*1, *6, *28,*60 and so on) in *UGT*1A1 and the functional characterization of known variants are helpful in elucidating the role of *UGT*1A1 genetic variation in irinotecan toxicity (18). The FDA has approved a *UGT*1A1 molecular assay test to detect polymorphisms in the *UGT*1A1 gene in clinical practice, so that patients with particular *UGT*1A1 gene variations that raise the risk of certain adverse effects can receive safer doses of irinotecan. This assay is intended to aid physicians to make decisions for individualized patient. Nevertheless, other important factors that affect dosing should also be considered, because severe toxicity sometimes occurs even in patients without particular *UGT*1A1 gene variations that place them at risk.

The *UGT*1A1 enzyme is responsible for hepatic bilirubin glucuronidation. A polymorphism in the *UGT*1A1 promoter has been linked with reduced *UGT*1A1 expression and is consequently associated with familiar hyperbilirubinemia. Accordingly, bilirubin levels may be associated with *UGT*1A1 function. The PTB level may reflect the total function of some polymorphisms in the *UGT*1A1 region and may be used as a simple and available surrogate marker for *UGT*1A1 function.

Recent studies have revealed that two major hepatic UGT, *UGT*1A1 and *UGT*1A9, and extra-hepatic *UGT*1A7 are involved in SN-38 glucuronidation (SN-38G) (7,19). The

Table 4. Univariate analysis of association between grade 3–4 diarrhea and pre-treatment clinical variables

	Diarrhea grade		Odds ratio (95% CI)
	Grade 0–2 ($n = 114$)	Grade 3–4 ($n = 13$)	
Sex			
Male	84	9	1
Female	30	4	1.24 (0.36–4.34)
Age			
Median (range)	65 (24–74)	65 (53–73)	1.07 (0.99–1.16)
Performance status			
0	29	5	1
1, 2	85	8	0.55 (0.17–1.80)
Liver metastasis			
No	101	8	1
Yes	13	5	4.86 (1.38–17.1)
Prior chemotherapy			
No	99	11	1
Yes	15	2	1.20 (0.20–7.04)
Treatment schedule			
Every 3 weeks	50	6	1
Every 4 weeks	64	7	0.91 (0.29–2.88)
Cisplatin dose (mg/m^2)			
60	66	5	1
80	48	8	2.20 (0.68–7.14)
AST (IU/l)			
Median (range)	21 (11–161)	23 (15–65)	1.00 (0.98–1.03)
ALT (IU/l)			
Median (range)	17 (5–266)	21 (14–84)	1.01 (0.99–1.02)
PNC ($\times 10^9$/l)			
Median (range)	4.2 (1.8–8.5)	3.5 (2.2–5.2)	0.77 (0.49–1.20)
PTB (mg/dl)			
Median (range)	0.55 (0.2–2.4)	0.6 (0.4–1.1)	1.95 (0.29–13.2)
≤ 0.7	96	11	1
>0.7	18	2	0.97 (0.20–4.75)

AST, aspartate aminotransferase; ALT, alanine aminotransferase; PNC, pre-treatment neutrophil count; PTB, pre-treatment total bilirubin.

efficacy of irinotecan is possibly affected by the activity of these genes. Thus, the product of some genetic polymorphisms in several genes may be a better pharmacogenetic marker for selecting patients who may not respond favorably to irinotecan-containing chemotherapy.

Cisplatin and irinotecan therapy is a standard regimen for both advanced non-small cell and small cell lung cancer (4). A randomized trial of irinotecan with or without cisplatin in patients with non-small cell lung cancer showed that grade 4 neutropenia was observed more frequently in the cisplatin–irinotecan arm (37%) than in the irinotecan-alone arm (8%), whereas grade 3 and 4 diarrhea was observed at the same frequency in both arms. In the present study, a higher cisplatin dose was associated with both grade 4 neutropenia and grade 3 and 4 diarrhea. The addition of cisplatin to another anti-cancer agent aggravated diarrhea in phase III studies (20), although diarrhea was moderate in cisplatin monotherapy observed in clinical trials (21). Thus, a higher dose of cisplatin seems to be associated with diarrhea, but the mechanism for this association remains unclear.

In this study PTB level was associated with the severity of neutropenia, but not with severity of diarrhea. When SN-38G is excreted in the bile and intestines, the bacteria-derived enzyme beta-glucuronidase converts SN-38G back