48. Tirelli U, Zagonel V, Serraino D, et al: Non-Hodgkin's lymphomas in 137 patients aged 70 years or older: A retrospective European Organization for Research and Treatment of Cancer Lymphoma Group Study. J Clin Oncol 6:1708-1713, 1988

49. Minami H, Sasaki Y, Saijo N, et al: Indirect-response model for the time course of leukopenia with anticancer drugs. Clin Pharmacol Ther 64:511-521, 1998

50. Minami H, Sasaki Y, Watanabe T, et al: Pharmacodynamic modeling of the entire time course of leukopenia after a 3-hour infusion of paclitaxel. Jpn J Cancer Res 92:231-238, 2001

51. Fliedner TM, Cronkite EP, Killmann SA, et al: Granulocytopoiesis II. Emergence and pattern of labeling of neutrophilic granulocytes in humans. Blood 24:683-700, 1964

52. Rothstein G, Christensen RD, Nielsen BR: Kinetic evaluation of the pool sizes and proliferative response of neutrophils in bacterially challenged aging mice. Blood 70:1836-1841, 1987

53. Timaffy M: A comparative study of bone marrow function in young and old individuals. Gerontol Clin (Basel) 4:13-18, 1962

54. Selig C, Nothdurft W: Cytokines and progenitor cells of granulocytopoiesis in peripheral blood of patients with bacterial infections. Infect Immun 63:104-109, 1995

Cancer Chemother Pharmacol (2004) 53: 129–132
DOI 10.1007/s00280-003-0707-8

ORIGINAL ARTICLE

Kiyoshi Mori · Yukari Kamiyama · Tetsuro Kondo
Yasuhiko Kano · Keigo Tominaga

Phase II study of the combination of vinorelbine and cisplatin in advanced non-small-cell lung cancer

Received: 14 April 2003 / Accepted: 31 July 2003 / Published online: 7 November 2003

Abstract *Purpose*: To evaluate the efficacy and safety of combination chemotherapy with cisplatin and vinorelbine for the treatment of previously untreated patients with advanced non-small-cell lung cancer (NSCLC). *Patients and methods*: Eligible patients were those with measurable NSCLC. They were treated with two or more cycles of a regimen consisting of vinorelbine 25 mg/m^2 on days 1 and 8 and cisplatin 80 mg/m^2 on day 1 every 3 weeks. *Results*: A total of 45 patients were enrolled. The response rate was 51.1% (23/45; 95% CI 35.8% to 66.3%). The median survival was 286 days with a 1-year survival rate of 40%. The median number of treatment cycles was 2. The major toxic effect was neutropenia of grade 3 or higher (84%). Nonhematological toxicities, including vomiting (62%), were mild (grade 2 or less). There were no treatment-related deaths. *Conclusion*: The high response rate and good tolerability proved this combination therapy to be a safe and effective treatment for advanced NSCLC.

Keywords Non-small-cell lung cancer · Vinorelbine · Cisplatin · Phase II study

Introduction

Vinorelbine ditartrate [1], a vinca alkaloid derivative, shows antitumor activity mainly by inhibiting microtubule polymerization in tumor cells just as other vinca alkaloid drugs do [2, 9]. Clinical studies of vinorelbine (VNR) have shown a good therapeutic outcome in non-small-cell lung cancer (NSCLC) and breast cancer, and a reduction in peripheral neuropathy that occurs frequently with vinca alkaloids [5, 7, 10, 12]. The combination of VNR and cisplatin (CDDP) (VP therapy) has shown a synergistic effect in vitro, while the main side effects are different between the drugs [4]. A phase I-II study has demonstrated efficacy of this combination in NSCLC [3]. VP therapy is considered a promising combination regimen for NSCLC on account of its higher response rate and longer survival compared with VNR or CDDP alone, or CDDP combined with vindesine [8, 17].

In clinical studies performed in Europe and the US, patient compliance rate was as low as 50% or less with regard to VNR when VP therapy, as VNR 25 mg/m^2 weekly and CDDP 80 mg/m^2 on day 1, was repeated every 4 weeks. This indicates the need to reconsider the dosing schedule of VNR [17]. Another dosing schedule for VP therapy (VNR 20 to 30 mg/m^2 on days 1 and 8 and CDDP 80 mg/m^2 on day 1 every 3 weeks) showed almost complete compliance and was found to be beneficial since the response rate was 28.3% to 56.7% and the survival 9.2 to 10.6 months [6, 13, 15, 17].

VP therapy is an effective regimen against advanced NSCLC. A multicenter joint phase III study is being planned in Japan to compare four regimens for advanced NSCLC: CDDP plus irinotecan used as a reference arm, CDDP plus VNR every 3 weeks, CDDP plus gemcitabine and carboplatin plus paclitaxel. A phase II study of VP therapy has not been conducted in Japan. We therefore carried out a phase II study of VNR 25 mg/m^2 on days 1 and 8 plus CDDP 80 mg/m^2 on day 1 given every 3 weeks in advanced NSCLC to evaluate the efficacy and safety of VP therapy.

Patients and methods

Patient selection

Patients eligible for the study were those admitted to our hospital between August 1999 and October 2001 who were histologically or

This work was supported in part by a grant-in-aid from the Ministry of Health and Welfare (Tokyo, Japan) and from the Second Term Comprehensive 10-Year Strategy for Cancer Control.

K. Mori (✉) · Y. Kamiyama · T. Kondo · Y. Kano
K. Tominaga
Department of Thoracic Diseases, Tochigi Cancer Center,
4-9-13 Yonan, Utsunomiya, 320-0834 Tochigi,
Japan
E-mail: kmori@tcc.pref.tochigi.jp
Tel.: +81-28-6585151
Fax: +81-28-6585669

cytologically diagnosed as having NSCLC and who were in clinical stage III or IV with unresectable disease, or in whom radiotherapy with curative intent was not possible, including those who had pleural effusion and dissemination, those with intrapulmonary metastasis within the ipsilateral lobe, those in whom the irradiation field exceeded one-half of one lung, those with metastasis to the contralateral hilar lymph nodes, and those with reduced lung function. None of the patients had received prior therapy. Other eligibility criteria included expected survival of 12 weeks, age ≤ 75 years, Eastern Cooperative Oncology Group performance score (PS) of 0–2, measurable lesions, adequate hematological function (WBC ≥4000/mm^3, platelet count ≥100,000/mm^3, hemoglobin ≥10 g/dl), renal function (serum creatinine ≤ 1.5 mg/dl, creatinine clearance ≥60 ml/min), and hepatic function (total serum bilirubin ≤ 1.5 mg/dl, serum GOT and serum GPT less than twice the upper limit of normal). Written informed consent was obtained from every patient with the statement that the patient was aware of the investigational nature of this treatment regimen. Pretreatment evaluation included medical history, physical examination, complete blood count, serum biochemical analyses, chest roentgenogram, electrocardiogram and urinalysis. All patients underwent radionuclide bone scan and computerized tomography of the brain, thorax, and abdomen.

Treatment

The anticancer drugs were administered via the intravenous route, VNR 25 mg/m^2 (Navelbine, Kyowa Hakko Kogyo) on days 1 and 8 and CDDP 80 mg/m^2 (Randa, Nippon Kayaku) on day 1. This combination therapy repeated every 3 weeks constituted a cycle of treatment. The minimal number of cycles to be evaluated was two. On day 8, the physician examined the patient and evaluated the development of adverse events, and if leukocytes had decreased to below 2000/mm^3, platelets had decreased to below 75,000/mm^3 or fever with infection had occurred, administration of VNR on that day was withheld at the discretion of the physician. To proceed with the second and subsequent cycles, patients were required to have a neutrophil count ≥1500/mm^3 and a platelet count ≥100,000/mm^3. Those patients receiving granulocyte colony-stimulating factor (G-CSF) were observed for 3 days after the final dose of G-CSF to ensure that their neutrophil count was 1500/mm^3 or more. Serum creatinine levels were required to be below the upper limit of normal and serum GOT/GPT levels below twice the upper limit of normal. In the presence of liver dysfunction due to apparent liver metastasis, however, serum GOT and GPT levels were required to be below three times the upper limit of normal. If fever occurred or if the PS advanced to grade 3 or worse, the subsequent cycle was postponed until the temperature fell below 38°C or until the PS returned to 2 or less. In the presence of grade 2 peripheral neuropathy dosing was temporarily postponed; with improvement to grade 1 or less treatment was cautiously resumed, but medication was discontinued if 6 weeks passed without any improvement. Peripheral neuropathy (including transient) grade 3 or higher required discontinuation of treatment. For the third and subsequent cycles, VNR or CDDP was decreased by 25% in accordance with the treatment-related adverse events observed during the preceding cycle. Steroid and HT_3-antagonist were administered to prevent nausea and vomiting.

Target population size and interim analysis

Simon's two-stage minimax design [16] was used to estimate the number of patients required for interim and final analyses at a threshold response rate (P_0) of 0.20, an expected response rate (P_1) of 0.40, $\alpha = 0.05$ and $\beta = 0.10$. If the interim analysis revealed 6 responding patients out of 24, recruitment would be continued until the target population size was achieved. The combination therapy was considered effective if 14 or more of 45 patients showed response in the final analysis.

Since an interim response rate of 48.1% (13/27) [11] was obtained, it was necessary to enroll up to 45 patients for the final analysis.

Evaluation of response and toxicity

Response and toxicity were evaluated on the basis of tumor images obtained by CT and other techniques, laboratory data and subjective/objective symptoms before, during and after administration of the study drugs and during the period from completion of treatment to the final analysis. Measurable disease parameters were determined every 4 weeks by various means such as computerized tomography. Evaluation was made in compliance with Response Evaluation Criteria in Solid Tumors (RECIST) guidelines [14] for antitumor activity and with NCI Common Toxicity Criteria version 2 for safety. The Institutional Ethical Review Committee gave approval to the study.

Results

Patient characteristics

Table 1 gives characteristics of the patients included. Their median age was 59.5 years (range 35 to 75 years). Male, PS 1 and adenocarcinoma predominated. There were 26 patients (58%) with stage IV disease and 19 (42%) with stage IIIB disease.

Treatments administered

The total number of cycles administered was 126 with a median of two per patient (ranging from one to four cycles; Table 2) and 43 patients received two cycles or more. In the two patients who received fewer than two cycles, treatment was discontinued because of CDDP-induced renal dysfunction in one and patient refusal in the other. Patients who completed two cycles or more accounted for 96% of patients (43/45). Except the two patients who received only one cycle, the every-3-week

Table 1 Patient characteristics

Eligible patients (*n*)	45
Age (years)	
Median	59.5
Range	35–75
Sex (*n*)	
Male	34
Female	11
Performance status (*n*)	
0	11
1	32
2	2
Histology (*n*)	
Adenocarcinoma	30
Squamous cell carcinoma	9
Other	6
Stage (*n*)	
IIIB	19
IV	26

Table 2 Efficacy of treatment ($n=45$)

No. of cycles	
Median	2.0
Range	1–4
Response	
Partial response	23
No change	21
Not evaluable	1
Response Rate (%)	51.1
95% CI (%)	35.8–66.3
1-year survival rate (%)	40

dosing schedule was adhered to by 88% of patients (38/43) in the second cycle, 68% (17/25) in the third and 92% (12/13) in the fourth, with a total of 83% (67/81). Only in two cycles was VNR withheld on day 8. The dose of VNR was reduced in 9% of dose administrations (22/250) and the does of CDDP was reduced in 8% (10/126). The planned dose intensities were 16.7 mg/m^2 per week for VNR and 26.7 mg/m^2 per week for CDDP while the actual dose intensities were 16.4 and 24.7 mg/m^2 per week, respectively. The median delivered dose intensity for CDDP (day 1) and VNR (days 1 and 8) of each course together was 90% or more (Table 3).

Efficacy of treatment

Of the 45 patients, 23 showed a partial response, 21 showed no change and 1 was not evaluable (Table 2). The response rate was 51.1% (23/45; 95% CI 35.8% to 66.3%; Table 2). The nonevaluable patient died of sudden hemoptysis on the 22nd day after the start of the second cycle (43rd day after the start of treatment) and could not be evaluated. Ten patients were alive at the time of this report. The time to progressive disease was 172 days and the median survival was 286 days (95% CI 248 to 404 days; Table 2). The 1-year survival rate was 40%.

Table 3 Median delivered dose intensity

	Median dose intensity (%)			
	Course 1	Course 2	Course 3	Course 4
CDDP	100	98.8	96	92.3
VNR				
Day 1	100	98.6	95.5	93.8
Day 8	97.8	98.6	95.5	93.8

Toxicities

Table 4 lists toxicities observed during the study. Hematological and blood biochemical reactions included a high incidence of leukopenia and neutropenia, i.e. leukopenia and neutropenia of grade 3 or higher occurred in 73% of patients (33/45) and 84% (38/45), respectively. Neutropenia-associated fever was limited to two patients. All neutropenic patients recovered upon treatment with G-CSF. Platelets decreased in 4% of patients (2/45). Creatinine was temporarily elevated in 15.6% (7/45).

Subjective and objective symptoms observed were of grade 2 or less and included vomiting in 77.8% of patients (35/45), hiccough in 33.3% (15/45), constipation in 40% (18/45), diarrhea in 22% (10/45), rash in 31.1% (14/45) and injection site reaction in 26.7% (12/45). All of these toxicities disappeared or improved with symptomatic treatment. There were no toxic deaths.

Discussion

As for the VP regimen for advanced NSCLC, the every-3-week dosing schedule has been tried in several medical facilities [6, 13, 15, 17]. Table 5 summarizes the clinical outcomes of every-3-week VP therapy reported in the literature and in this study. Response rates range from 28% to 57% and median survival is approximately 10 months. The results are similar among the studies.

In 96% of patients (43/45), two or more cycles of VP therapy were administered. The every-3-week dosing

Table 4 Toxicities ($n=45$)

Toxicity	Grade (Common Toxicity Criteria)				Grade 3/4 (%)
	1	2	3	4	
Leukopenia	4	3	25	8	33 (73%)
Neutropenia	2	2	13	25	38 (84%)
Anemia	12	3	1	4	5 (11%)
Thrombocytopenia	5	1	2	0	2 (4%)
Creatinine	5	2	0	0	–
Vomiting	29	6	0	0	–
Hiccough	15	0	0	0	–
Constipation	13	5	0	0	–
Diarrhea	9	1	0	0	–
Rash	10	4	0	0	–
Neuropathy	4	0	0	0	–
Injection site reaction	4	8	0	0	–
Alopecia	3	0	0	0	–

Table 5 Outcomes of studies of VP therapy (VNR days 1 and 8, CDDP day 1, every 3 weeks)

Reference	VNR (mg/m^2)	CDDP (mg/m^2)	Response	Median survival time (months)
4	25	80	28.3% (28/99)	9.2
10	25	80	56.7% (42/74)	10
11	20–25	80	46.7% (14/30)	10.6
1	30	80	36.2% (47/130)	–
Present study	25	80	51.1% (23/45)	9.6

schedule was adhered to in 85% of all cycles administered. In cycles in which noncompliance was seen, medication was postponed to the 4th to 5th week because, in most cases, the neutrophil count in the 3rd week failed to meet the criterion for going on to subsequent cycles. The planned dose intensity was almost attained since the actual dose intensity was 16.4 mg/m^2 per week for VNR and 24.7 mg/m^2 per week for CDDP, accounting for 98% and 93% of the planned values, respectively [13].

Most adverse reactions were hematological. In particular, leukopenia and neutropenia of grade 3 or worse occurred in 73% and 84% of 45 patients, respectively. Others have reported the incidence of leukopenia of grade 3 or worse to be 8% to 33% [6, 13, 17]. Although the difference in patient characteristics hinders simple comparison and analysis of these data, it can be said that leukopenia was more frequent in our study. The leukocyte count improved rapidly upon treatment with G-CSF. Nonhematological toxicities were mild and adverse reactions of grade 3 or higher were not noted.

The combination of VNR 25 mg/m^2 on days 1 and 8 and CDDP 80 mg/m^2 on day 1 was administered every 3 weeks to 45 patients with advanced NSCLC in this phase II study. The response rate was 51.1%; the main adverse effect was neutropenia. The high response rate and good tolerability indicate that this combination therapy is a safe and effective treatment for advanced NSCLC. Its usefulness will be further verified in phase III studies.

References

1. Adam Z, Coupkova H, Kolek V, et al (1995) Vinorelbine and cisplatin in the treatment of advanced non-small-cell lung cancer: results of a multicenter Czech study. Acta Med Austriaca 22:120–124
2. Berthaud P, Le Chevalier LT, Ruffie P, et al (1992) Phase I-II study of vinorelbine (Navelbine) plus cisplatin in advanced non-small cell lung cancer. Eur J Cancer 28A:1863–1865
3. Binet S, Fellous A, Lataste H, Krikorian A, Couzinier JP, Meininger V (1989) In situ analysis of the action of navelbine on various types of microtubules using immunofluorescence. Semin Oncol 16 [Suppl 4]:5–8
4. Bretti S, Berruti A, Gorzegno G, et al (1996) Multicenter phase II trial of intermediate dose cisplatin and vinorelbine in inoperable non-small cell lung cancer patients. Lung Cancer 14:353–360
5. Canobbio L, Boccardo F, Pastorino G, et al (1989) Phase II study of Navelbine in advanced breast cancer. Semin Oncol 16 [Suppl 4]:33–36
6. Chevalier TL, Brisgand D, Douillard JY, et al (1994) Randomized study of vinorelbine and cisplatin versus vindesine and cisplatin versus vinorelbine alone in advanced non-small cell lung cancer: results of a European multicenter trial including 612 patients. J Clin Oncol 12:360–367
7. Depierre A, Lemarie E, Dabouis G, Garnier G, Jacoulet P, Dalphin JC (1989) Efficacy of Navelbine (NVB) in non-small cell lung cancer (NSCLC). Semin Oncol 16 [Suppl 4]:26–29
8. Depierre A, Lemarie E, Dabouis G, Garnier G, Jacoulet P, Dalphin JC (1991) A phase II study of Navelbine (vinorelbine) in the treatment of non-small cell lung cancer (NSCLC). Am J Clin Oncol 14:115–119
9. Fellous A, Ohayon R, Vacassin T, et al (1989) Biochemical effects of Navelbine on tubulin and associated proteins. Semin Oncol 16 [Suppl 4]:9–14
10. Frontini L, Candido P, Cattaneo MT, et al (1996) Cisplatin-vinorelbine combination chemotherapy in locally advanced non-small cell lung cancer. Tumori 82:57–60
11. Gebbia V, Caruso M, Valenza R, et al (1994) Vinorelbine plus cisplatinum for the treatment of stage IIIB and IV non-small cell lung carcinoma. Anticancer Res 14:1247–1250
12. Gomi K, Ohno H, Nomura K, Okabe M, Kobayashi K, Niitani H (1986) Kinetic analysis of combination effect of Navelbine (KW-2307) with cisplatin against human lung adenocarcinoma PC-12 cell in culture. Jpn J Cancer Res 83:532–539
13. Kusunoki Y, Furuse K, Yamori S, et al (1995) Randomized phase II study of vinorelbine (VRB) vs vindesine (VDS) in previously untreated non-small cell lung cancer (NSCLC) (abstract 1071). Proc Am Soc Clin Oncol 14:353
14. Mori K, Kamiyama Y, Kondoh T, Tominaga K (2002) Phase II study of combined vinorelbine plus cisplatin for advanced non-small cell lung cancer (an interim report). Jpn Cancer Chemother 29:405–410
15. Potier P (1989) The synthesis of navelbine prototype of a new series of vinblastine derivatives. Semin Oncol 16 [Suppl 4]:2–4
16. Simon R (1989) Optimal two-stage designs for phase II clinical trial. Control Clin Trial 10:1–10
17. Wozniak AJ, Crowley JJ, Balcerzak SP, et al (1998) Randomized trial comparing cisplatin with cisplatin plus vinorelbine in the treatment of advanced non-small-cell lung cancer: a Southwest Oncology Group study. J Clin Oncol 16:2459–2465

Cancer Chemother Pharmacol (2003) 51: 403–406
DOI 10.1007/s00280-003-0585-0

ORIGINAL ARTICLE

Kiyoshi Mori · Tetsuro Kondo
Yukari Kamiyama · Yasuhiko Kano
Keigo Tominaga

Preventive effect of Kampo medicine (Hangeshashin-to) against irinotecan-induced diarrhea in advanced non-small-cell lung cancer

Received: 1 April 2002 / Accepted: 15 January 2003 / Published online: 9 April 2003

Abstract *Purpose*: Kampo medicine Hangeshashin-to (TJ-14) which contains baicalin, a β-glucuronidase inhibitor, alleviates diarrhea induced by irinotecan (CPT-11). We conducted a randomized comparative trial to investigate whether support with TJ-14 would prevent and control CPT-11-induced diarrhea. *Methods*: Of 44 previously untreated patients with advanced non-small-cell lung cancer randomized, 41 (18 TJ-14 group, 23 control group) were available for evaluation. The chemotherapy regimen consisted of a combination of cisplatin and CPT-11. TJ-14 (7.5 g/day) was administered orally. *Results*: Of the 41 patients, 39 experienced diarrhea. Compared with the control group, the TJ-14 group showed a significant improvement in diarrhea grades ($P=0.044$) as well as a reduced frequency of diarrhea grades 3 and 4 (one patient versus ten patients; $P=0.018$). However, the two groups showed no differences in the frequency of diarrhea or the number of days the symptoms continued. This study was stopped at an interim evaluation. *Conclusion*: TJ-14 was effective in preventing and controlling CPT-11-induced diarrhea.

Keywords Non-small-cell lung cancer · Irinotecan · Diarrhea · Kampo medicine · Hangeshashin-to

This work was supported in part by a Grant-in Aid for Cancer Research from the Ministry of Health, Labour and Welfare, the Foundation for the Promotion of Cancer Research, and the Second Term Comprehensive 10-Year Strategy for Cancer Control.

K. Mori (✉) · T. Kondo · Y. Kamiyama · Y. Kano
K. Tominaga
Department of Thoracic Diseases, Tochigi Cancer Center,
4-9-13 Yonan, Utsunomiya, 320 Tochigi, Japan
E-mail: kmori@tcc.pref.tochigi.jp
Tel.: +81-286-585151
Fax: +81-286-585669

Introduction

Irinotecan hydrochloride (CPT-11), a semisynthetic derivative of camptothecin, is an anticancer drug which inhibits nucleic acid synthesis by topoisomerase I inhibition [7, 9]. CPT-11 possesses a wide antitumor spectrum, and has been proven to have antitumor effects against lung cancer [4, 8, 13, 14], colon cancer [17, 19] and malignant lymphoma [15]. At present, it is used in a large number of institutions in combination with other drugs [11, 16]. The leading side effects of CPT-11 include leukopenia and diarrhea. These side effects are the main reason for discontinuing administration, and the drug thus has dose-limiting toxicity [4, 13]. The reduction in leukocytes is usually improved by the administration of recombinant human granulocyte colony-stimulating factor (rG-CSF) [11, 16].

Studies of CPT-11 in monotherapy and combination therapy regimens have shown that diarrhea occurs in 63–79% of the patients [1, 4, 15, 16, 19]. There are two types of CPT-11-induced diarrhea: acute diarrhea that occurs during the early stages of drug administration, and delayed diarrhea that occurs during later stages. The former is thought to be caused by the anticholinesterase actions of CPT-11 [5]. It is mostly transient in nature, and can be treated effectively with anticholine drugs [17]. The latter, in contrast, can sometimes develop into severe diarrhea. Symptoms in some patients can become difficult to control, while other patients become unable to continue taking CPT-11 [17]. The following drugs are used in several institutions to treat diarrhea clinically: anticholine drugs [17], loperamide hydrochloride [1, 17], and Kampo medicine Hangeshashin-to (TJ-14) [18]. Animal experiments have shown that TJ-14 effectively prevents CPT-11-induced delayed diarrhea [20].

We conducted a randomized comparative trial to investigate whether support with TJ-14 would prevent and control CPT-11-induced-diarrhea in combination therapy with cisplatin and CPT-11 in advanced non-small-cell lung cancer (NSCLC).

Subjects and methods

Patient eligibility

Subjects entered into the study were NSCLC patients attending our hospital who underwent combination therapy with cisplatin and CPT-11. None of the patients had received prior therapy. Other eligibility criteria included an expected survival of at least 12 weeks, age ≤ 75 years, Eastern Cooperative Oncology Group (ECOG) performance status (PS) 0–2, and adequate hematological function (WBC ≥4000/mm^3, platelet count ≥100,000/mm^3, hemoglobin ≥10 g/dl), renal function (serum creatinine ≤ 1.5 mg/dl, creatinine clearance ≥60 ml/min) and hepatic function (total serum bilirubin ≤ 1.5 mg/dl, glutamic oxaloacetic transaminase and glutamic pyruvic transaminase less than twice the upper limit of the normal range). This protocol was approved by the ethical committee of the Tochigi Cancer Center. Written informed consent was obtained from every patient stating that the patient was aware of the investigational nature of this treatment regimen. Patients with severe complications, diarrhea, massive pleural and cardiac effusion, and symptomatic brain metastasis were excluded from the study. Pretreatment evaluation included medical history, physical examination, complete blood count, serum biochemical analysis, chest roentgenogram, electrocardiogram, and urinalysis. All patients underwent a radionuclide bone scan, computerized tomography of the brain and thorax, and computerized tomography of the abdomen. All patients were admitted to the Tochigi Cancer Center Hospital during this trial. Complete blood count, biochemical tests, serum electrolytes, urinalysis, and chest roentgenograms were obtained weekly. Tests of measurable disease parameters such as computerized tomography were repeated every 4 weeks. Staging was according to the 4th edition of the UICC TNM classification.

TJ-14 administration

After informed consent had been obtained, patients were randomized using random numbers to receive either TJ-14 or not. Patients in the TJ-14 group were given a total of 7.5 g TJ-14 (Tsumura Company, Tokyo) three times a day before each meal in equal doses, beginning more than 3 days before the start of chemotherapy. The patients received TJ-14 for a minimum of 21 consecutive days after the start of chemotherapy. When the chemotherapy was administered for two or more courses, TJ-14 was administered following a similar schedule. However, the administration of TJ-14 after the second course was optional. Proper treatment was provided to patients with severe diarrhea, comprising loperamide hydrochloride for diarrhea grade 2 or higher under the common toxicity criteria (CTC) (version 2.0), and codeine phosphate and electrolyte parenteral fluid for grade 3 or 4 diarrhea.

Evaluation

The antidiarrheic responses to and adverse effects of TJ-14 were evaluated based on records kept by the patients and medical staff. Items recorded daily included the features and frequency of stool, presence and degree of abdominal pain during bowel movement, presence of night-time bowel movement, and presence of hemorrhagic diarrhea. Diarrhea was judged in accordance with the CTC criteria. Evaluation of diarrhea for both the TJ-14 group and the control group was made during the first course.

Study design

With regard to sample size calculations, the frequency of grade 3 diarrhea in the control group was estimated as 30%, and the frequency of diarrhea in the TJ-14 group as 10%. The number of subjects needed per group was estimated to be 50 under the conditions $\alpha = 0.05$, $\beta = 0.20$, by approximation to normality of the estimated difference in the frequency of the subjects who develop diarrhea. Therefore, the target number of subjects for each group was set at 60 in consideration of the presence of ineligible and nonassessable cases.

Interim evaluation was performed twice, i.e. when 20 and 40 subjects of each group became assessable. The efficacy and safety evaluations were performed on the basis of the results of these interim evaluations. With regard to the evaluation of efficacy and safety in the interim analysis, the study was to be terminated if a statistically significant between-group difference in the incidence of diarrhea of grade 3 or greater was observed. If no statistically significant difference was present, the study was to continue. In the final analysis, TJ-14 was to be evaluated as effective if a significant decrease in the incidence of diarrhea of grade 3 or greater was observed in the TJ-14 group.

To determine the significance of differences, the Wilcoxon rank-sum test, Student's t-test, and Fisher's χ^2 test were used in accordance with the nature of the variables.

Chemotherapy

The anticancer drug regimen consisted of combined CPT-11 plus infusional cisplatin with rG-CSF support. A dose of 160 mg/m^2 of CPT-11 (Daiichi Pharmaceutical Company, Japan) in 500 ml normal saline or 5% glucose was infused intravenously (i.v.) over 90 min on day 1, and 20 mg/m^2 of cisplatin was given daily for 5 days by continuous i.v. infusion. One-third of the daily dose was administered every 8 h dissolved in 800 ml physiological saline [11]. rG-CSF (Chugai Pharmaceutical Company, Japan) was administered subcutaneously at a dose of 2 μg/kg for, in principle, 16 days (days 6 to 21), beginning on the day after of completion of cisplatin administration, once every day, at the same time whenever possible. However, if the granulocyte count increased to more than 5000/mm^3 or the white blood count increased to more than 10,000/mm^3 after reaching a nadir, administration was discontinued. The course was repeated every 4 weeks. The antiemetic drugs used were metoclopramide (3 mg/kg per day, continuous infusion for 5 days), methylprednisolone (125 mg bolus infusion every 8 h, days 1–5), diphenhydramine (30 mg orally, days 1–7) and alprazolam (1.2 mg orally, days 1–7). The dose of CPT-11 was reduced to 120 mg/m^2 for the subsequent course if grade 3 or 4 diarrhea occurred.

Results

We performed an interim analysis. Of 44 patients randomized, 3 requested cancellation of oral administration of TJ-14 prior to the start of chemotherapy. Therefore, 41 patients were evaluable, 18 in the TJ-14 group and 23 in the control group. Table 1 shows the patient characteristics. Compared with the TJ-14 group, the control group had a slightly higher percentage of female, PS 2, stage III, and squamous cell carcinoma patients, although differences between the two groups were not significant. The TJ-14 group received an average 2.4 courses (1–4) of chemotherapy, and the control group, an average 2.5 courses (1–5). All except two patients in the TJ-14 group (i.e. 39 patients, 95%) had diarrhea. Diarrhea occurred on average 6.3 days after the start of chemotherapy in the TJ-14 group (range 1–11 days) and 5.9 days in the control group (range 1–11 days). The day on which diarrhea occurred most frequently was on average 9.2 days after the start of chemotherapy in the TJ-14 group (range 1–14 days) and 9 days (range 1–16 days) in the control group.

Table 1 Patient characteristics (values are number of patients, except age in years)

	TJ-14 group	Control group	*P* value
Total no. of patients	18	23	
Age (years)			0.834
Mean	61.2	60.4	
Range	37–75	44–74	
Sex			0.308
Male	15	16	
Female	3	7	
Performance status			0.800
0	2	3	
1	14	16	
2	2	4	
Stage			0.194
III	3	8	
IV	15	15	
Histological type			0.595
Adenocarcinoma	14	14	
Squamous cell carcinoma	4	8	
Adenosquamous	0	1	

Table 2 Evaluation of diarrhea

	TJ-14 group	Control group	*P* value
CTC grade (no. of patients)			0.044
0	2	0	
1	5	7	
2	10	6	
3	1	6	
4	0	4	
3 + 4	1	10	0.018
Frequency			
Mean	2.39	3.52	0.17
Total	204	515	0.32
Duration (days)			0.58
Mean	4.4	4.7	
Total	80	109	

Table 2 shows the evaluations of diarrhea in the TJ-14 group and the control group during the first course of chemotherapy. Compared with the control group, the TJ-14 group showed significantly improved grades of diarrhea ($P=0.044$) and had a significantly lower incidence of diarrhea grades 3 and 4 ($P=0.018$). However, no differences were seen between the two groups in terms of frequency of diarrhea or the number of days the symptoms continued.

After the second course and beyond, 6 out of 15 patients in the TJ-14 group did not receive TJ-14, while 2 out of 20 patients in the control group received TJ-14. With regard to changes in the grade of diarrhea during the course before and after TJ-14 administration, there was a change in only one patient in the TJ-14 group (from grade 1 to grade 2), while in the control group, one patient improved from grade 3 to grade 1. No serious side effects of TJ-14 were seen except for two patients who had grade 1 constipation. Thus, TJ-14 significantly reduced the time to onset of CPT-11-induced diarrhea. The study was stopped at an interim evaluation.

Discussion

Diarrhea of grade 3 or greater associated with anticancer drugs has been observed in 2% of patients receiving chemotherapy comprising cisplatin (bolus) and vindesine [3], while the corresponding figure in patients receiving a combination of cisplatin (continuous infusion) and vindesine was 17% [10]. The patients in these two studies cannot be compared without qualification because of differences in the patient background factors and dosage of cisplatin; nevertheless, the incidence of diarrhea of grade 3 or greater is likely to be higher in patients receiving continuous infusion than in those receiving bolus cisplatin. Additionally, the incidence of diarrhea of grade 3 or greater has been reported to be 16% with combination chemotherapy comprising cisplatin (bolus, 60 mg/m^2; day 1) and CPT-11 (60 mg/m^2; days 1, 8 and 15) [14]. Hence, because the present regimen involved the administration of CPT-11 at a dose of 160 mg/m^2 on day 1 in addition to cisplatin (continuous infusion, 20 mg/m^2; days 1–5), the overall incidence of diarrhea of grade 3 or greater was high (27%; TJ-14 group 6%, control group 43%).

A combination of cisplatin and CPT-11 was administered to treat advanced NSCLC, and random comparative tests were carried out to determine the usefulness of TJ-14 for the prevention and control of diarrhea induced by CPT-11. We performed an interim analysis in 44 patients randomized. TJ-14 significantly reduced the onset of CPT-11-induced diarrhea. In the TJ-14 group, the drug improved the grade of diarrhea ($P=0.044$) and reduced the incidence of diarrhea grade 3 or 4 ($P=0.018$). Thus our study was stopped at an interim evaluation.

It has been reported that medicines such as anticholine agents [17] and loperamide hydrochloride [1, 17] are effective in preventing CPT-11-induced diarrhea. A high dose of loperamide hydrochloride (2 mg loperamide hydrochloride once every 2 h) has been shown to be particularly effective against delayed diarrhea (diarrhea occurring 8 h and more after CPT-11 administration) that accompanies high doses of CPT-11 (between 400 and 600 mg/m^2 of CPT-11 administered once every 3 weeks) [1].

The mechanism of CPT-11-induced delayed diarrhea is as follows. CPT-11 is changed to 7-ethyl-10-hydroxycamptothecin (SN-38) in the liver, and SN-38 undergoes glucuronate conjugation changing into inactive SN-38 glucuronide. Later, it is excreted into the bile, and is then deconjugated by β-glucuronidase contained in the intestinal bacteria to become SN-38 once again [6]. This SN-38 directly damages the intestinal mucous membrane and induces delayed diarrhea [2]. Therefore, it is considered possible to prevent CPT-11-induced delayed diarrhea by using a glucuronide inhibitor of β-glucuronidase [12]. Kampo medicine (TJ-14) contains components of baic-

alin which serve as this glucuronide. Experiments with rats have shown that it effectively prevents CPT-11-induced delayed diarrhea [20], Sakata et al. [18] have reported that TJ-14 can effectively prevent diarrhea caused by CPT-11. As a side effect of TJ-14, constipation occurred in 10% of patients in the study by Sakata et al. [18] and in 11% of patients in our study, although they were all mild grade 1.

Concerning comparative tests using Kampo medicine as the control drug, we were unable to make a placebo formulation because of the Kampo drug's distinct shape and smell, and therefore could not carry out double-blind comparative tests. As our study did not use a double-blind test method, the results may have been affected, to a certain extent, by bias on the part of the patients and physicians.

In conclusion, TJ-14 was able to alleviate CPT-11-induced diarrhea in advanced NSCLC. The clinical usefulness of TJ-14 must be studied further, based on our results, by administering the drug to prevent the onset of CPT-11-induced diarrhea and conducting a large-scale cooperative trial with other institutions.

References

1. Abigerges D, Armand J-P, Chabot GG, Costa LD, Fadel E, Cote C, Herait D, Gandia D (1994) Irinotecan (CPT-11) high-dose escalation using intensive high-dose loperamide to control diarrhea. J Natl Cancer Inst 86:446
2. Asumi R, Suzuki W, Hakusui H (1991) Identification of the metabolites of irinotecan, a new derivative of camptothecin, in rat bile and its biliary excretion. Xenobiotica 21:1159
3. Fukuoka M, Masuda N, Furuse K, Negoro S, Takada M, Matsui K, Takifuji N, Kudoh S, Kawahara M, Ogawara M, Kodama N, Kubota K, Yamamoto M, Kusunoki Y (1991) A randomized trial in inoperable non-small-cell lung cancer: vindesine and cisplatin versus mitomycin, vindesine and cisplatin versus etoposide and cisplatin alternating with vindesine and mitomycin. J Clin Oncol 9:606
4. Fukuoka M, Niitani H, Suzuki A, Motomiya M, Hasegawa K, Nishiwaki Y, Kuriyama T, Ariyoshi Y, Negoro S, Masuda N, Nakajima S, Taguchi T (1992) A phase II study of CPT-11, a new derivative of untreated non-small-cell lung cancer. J Clin Oncol 10:16
5. Gandia D, Abigerges D, Armand J-P, Chabot G, Costa LD, Forni MD (1993) CPT-11 induced cholinergic effects in cancer patients (letter). J Clin Oncol 11:196
6. Kaneda N, Nagata H, Furuta T, Yokokura T (1990) Metabolism and pharmacokinetics of the camptothecin analogue CPT-11 in mouse. Cancer Res 50:1715
7. Kunimoto T, Nitta K, Tanaka T, Uehara N, Baba H, Takeuchi M, Yokokura T, Sawada S, Miyasaka T, Mutai M (1987) Antitumor activity of 7-ethyl-10-[4-(1-piperidino)-1-piperidino] carbonyloxy-camptothecin, a novel water soluble derivative of camptothecin, against murine tumors. Cancer Res 47:5944
8. Masuda N, Fukuoka M, Kusunoki Y, Mastui K, Takifuji N, Kudoh S, Negoro S, Nishioka M, Nakagawa K, Takada M (1992) CPT-11: a new derivative of camptothecin, for the treatment of refractory or relapsed small-cell lung cancer. J Clin Oncol 10:1225
9. Matsuzaki T, Yokokura T, Mutai M, Tsuruo T (1988) Inhibition of spontaneous and experimental metastasis by a new derivative of camptothecin, CPT-11, in mice. Cancer Chemother Pharmacol 21:308
10. Mori K, Saito Y, Tominaga K (1992) Phase II study of cisplatin continuous infusion plus vindesine in the treatment of non-small-cell lung cancer. Am J Clin Oncol 15:344
11. Mori K, Hirose T, Machida S, Yokoyama K, Tominaga K (1997) A phase I study of irinotecan and infusional cisplatin with recombinant human granulocyte colony-stimulating factor support in the treatment of advanced non-small cell lung cancer. Eur J Cancer 33:503
12. Narita M, Nagai E, Hagiwara H, Aburada M, Yokoi T, Kamataki T (1993) Inhibition of β-glucuronidase by natural glucuronides of Kampo medicines using glucuronide of SN-38 (7-ethyl-10-hydroxycamptothecin) as a substrate. Xenobiotica 23:5
13. Negoro S, Fukuoka N, Masuda N, Takada M, Kusunoki Y, Matsui K, Takifuji N, Kudoh S, Niitani H, Taguchi T (1991) Phase I study of weekly administration of CPT-11. J Natl Cancer Inst 83:1164
14. Noda K, Nishiwaki Y, Kawahara M, Negoro S, Sugiura T, Yokoyama A, Fukuoka M, Mori K, Watanabe K, Tamura T, Yamamoto S, Saijo N (2002) Irinotecan plus cisplatin compared with etoposide plus cisplatin for extensive small-cell lung cancer. N Engl J Med 346:85
15. Ohno R, Okada K, Masaoka T, Kuramoto A, Arima T, Yoshida Y, Ariyoshi H, Ichimaru M, Sakai Y, Oguro M, Ito Y, Morishima Y, Yokomaku S, Ota K (1990) An early phase II study of CPT-11: a new derivative of camptothecin, for the treatment of leukemia and lymphoma. J Clin Oncol 8:1907
16. Oshita F, Noda K, Nishiwaki Y, Fujita A, Kurita Y, Nakabayashi T, Tobise K, Abe S, Suzuki S, Hayashi I, Kawakami Y, Matsuda T, Tsuchiya S, Takahashi S, Tamura T, Saijo N (1997) Phase II study of irinotecan and etoposide in patients with metastatic non-small-cell lung cancer. J Clin Oncol 15:304
17. Rothenberg ML, Kuhn JG, Burris HA III, Nelson J, Eckardt JR, Tristan-Morales M, Hilsenbeck SG, Weiss GR, Smith LS, Rodriguez GI, Rock MK, Von Hoff DD (1993) Phase I and pharmacokinetic trial of weekly CPT-11. J Clin Oncol 11:2194
18. Sakata Y, Suzuki H, Kamataki T (1994) Preventive effect of TJ-14, a Kampo (Chinese herb) medicine, on diarrhea induced by irinotecan hydrochloride (CPT-11) (in Japanese with English abstract). Jpn J Cancer Chemother 21:1241
19. Shimada Y, Yoshino M, Wakui A, Nakao I, Futatsuki K, Sakata Y, Kambe M, Taguchi T, Ogawa N (1993) Phase II study of CPT-11, a new camptothecin derivative, in metastatic colorectal cancer. J Clin Oncol 11:909
20. Takasuna K, Kasai Y, Kitano Y, Mori K, Kobayashi R, Hagiwara T, Kakihata K, Hirohashi M, Nomura M, Nagai E, Kamataki T (1995) Protective effects of Kampo medicine and baicaline against intestinal toxicity of a new anticancer camptothecin derivative, irinotecan hydrochloride (CPT-11), in rats. Jpn J Cancer Res 86:978

Int J Clin Oncol (2004) 9:184–188
DOI 10.1007/s10147-004-0399-z

ORIGINAL ARTICLE

Kiyoshi Nakamatsu · Yasumasa Nishimura
Minoru Suzuki · Shuichi Kanamori · Osamu Maenishi
Yoshiko Yasuda

Erythropoietin/Erythropoietin-receptor system as an angiogenic factor in chemically induced murine hepatic tumors

Received: June 2, 2003 / Accepted: February 27, 2004

Abstract
Background. To clarify the role of erythropoietin (Epo) in hepatic tumor angiogenesis, expression of Epo and its receptor (Epo-R) and content of Epo were investigated in murine chemically induced hepatic tumors.
Methods. To induce hepatic tumors and cirrhosis, diaminobenzidine was administered to Wistar rats for 5 months. In total, 30 hepatic tumors of greater than 3 mm in diameter were induced in 12 rats. The 30 hepatic tumors were resected with the surrounding hepatic tissues. The Epo content was measured by a radioimmunoassay (RIA) method. The number of tumor vessels in a definite area was counted in 100 areas of each tumor. To demonstrate the expression of Epo-R in tumors or surrounding liver tissues, immunohistochemial staining for Epo-R was performed.
Results. The Epo content of tumors ranged from 6.1 to 97.8 mU/ml, with a median of 21.8 mU/ml, which was significantly higher than that of the cirrhotic tissues adjacent to the tumors. Epo was not detectable in the normal or cirrhotic liver tissues without tumors. A significant correlation between Epo content and vascular density was noted in the 30 hepatic tumors (correlation coefficient, 0.480; $P = 0.01$). Immunoreactive Epo-R was detectable in the endothelium of intervening vessels of all hepatic tumors examined.
Conclusion. The Epo/Epo-R system is related to the angiogenesis of murine hepatic tumors.

Key words Erythropoietin · Erythropoietin receptor · Tumor angiogenesis · Hepatic tumors · Diaminobenzidine · Rats

K. Nakamatsu (✉) · Y. Nishimura · M. Suzuki · S. Kanamori
Department of Radiology, Kinki University School of Medicine, 377-2 Ohno-Higashi, Osaka-Sayama, Osaka 589-8511, Japan
Tel. +81-72-366-0221, ext 3132; Fax +81-72-368-2388
e-mail: kiyoshi@med.kindai.ac.jp

O. Maenishi
First Department of Pathology, Kinki University School of Medicine, Osaka, Japan

Y. Yasuda
First Department of Anatomy, Kinki University School of Medicine, Osaka, Japan

Introduction

Erythropoietin (Epo) is a glycoprotein with a molecular weight of 34000 daltons. Epo is a key factor for regulating erythropoiesis, by stimulating the proliferation and differentiation of late erythroid precursor cells. Hypoxia increases the production of Epo, and hyperoxia reduces the amount of Epo.[1,2] Epo is activated by binding to its specific receptor (Epo-R).[1] In adults, Epo is mainly produced by the kidney, although it is synthesized in the liver during fetal life.[3] However, recently, many extrarenal and extrahepatic production sites of Epo were reported, such as the brain,[2] embryos including the developing nervous system,[4] yolk sac,[5] uterus, and ovary.[6–9]

At these extrarenal and extrahepatic Epo production sites, Epo may play a role other than erythropoiesis. There is accumulated evidence that Epo has angiogenic activity;[3] Epo-R mRNA has been demonstrated in endothelial cells,[8,10,11] Epo stimulates the proliferation and migration of endothelial cells in vitro[12] and in vivo angiogenesis.[6,13,14]

In the mouse uterus, Epo is involved in 17β-estradiol (E2)-dependent cyclical uterine angiogenesis via Epo-R expressed in the vascular endothelial cells.[6] Recently, Yasuda et al.[8] demonstrated that inhibition of Epo signaling destroyed xenografts of ovarian and uterine cancers in nude mice.

There are several case reports that human hepatocellular carcinoma (HCC) and renal cell carcinoma (RCC) produced marked erythrocytosis and increased the levels of serum Epo or the production of Epo-like activity in cell cultures.[15,16] Because both HCC and RCC are hypervascular tumors and the liver and kidney are major Epo production sites, it is suggested that Epo signaling may contribute to the development and progression of malignant hepatic tumors. Thus, to clarify the role of Epo in the angiogenesis of he-

patic tumors, expression of Epo and Epo-R were investigated in chemically induced murine hepatic tumors.

Materials and methods

Animals and tumors

Male Wistar rats were purchased from Clea Japan (Tokyo, Japan) at 8 weeks of age. To induce hepatic tumors, 45 rats were placed (4 rats per each aseptic chamber) in a specific pathogen-free room, and given a carcinogenic diet containing 0.06% 3'-methyl-4-dimethylaminoazobenzene (3'-Me-DAB) for 5 months.[17] Development of hepatic tumors was examined by ultrasonography every weeks 5 months after the start of 3'-Me-DAB administration. A total of 30 tumors greater than 3 mm (range, 3–15 mm) in diameter were detected in 24 of the 45 rats, and used for the present analysis. In addition, 5 rats given sterilized normal pellets for 5 months were used as controls.

Deep anesthesia was induced in the rats with pentobarbital sodium (0.8 mg/kg), and only tumor tissues were resected, without the surrounding cirrhotic tissues, in the first 12 tumors that appeared in 12 rats. In the later analysis, hepatic tumors, with the surrounding hepatic tissues, were resected for the remaining 18 tumors in 12 rats. The adjacent cirrhotic tissues were sampled 3–7 mm from the edge of each tumor. The tumors with or without surrounding cirrhotic tissues were dissected into two pieces; half of the tumor was fixed in Zamboni solution for immunostaining and hematoxylin-eosin (H&E) staining, and the other was frozen in liquid nitrogen for determination of Epo content. In addition, five pieces of cirrhotic tissues without hepatic tumors in the same lobe and five pieces of normal liver tissues obtained from control rats were processed similarly.

Determination of Epo

The content of Epo was determined by a radioimmunoassay (RIA) method (Ohtuska Assay, Tokyo, Japan). Thirty pieces from all 30 tumors and the 18 pieces of the adjacent cirrhotic tissues were studied. The tissue blocks were homogenized, using phosphate-buffered saline (PBS) with 0.5% Tween 20 at five times the weight of the blocks. In addition, the content of Epo was determined for the five pieces of cirrhotic tissues without hepatic tumors in the same lobe and five pieces of normal liver tissues obtained from control rats.

Immunohistochemical staining for Epo-R

After cryprotection, the specimens were cut into 6-μm slices with a cryostat. The sections were stained by the labelled streptavidin biotin method, using a Vector ABC kit (Vector Laboratories, Burlingame, CA, USA). As the primary antibody against Epo-R, rabbit anti-mouse Epo-receptor polyclonal antibody was used (1:500 dilution). Control sections were stained with non-immune rabbit γ-globulin.

Vascular density

Specimens were stained with anti-Factor-VIII (Dako, Carpinteria, CA, USA; 1:200 dilution) to identify the vascular endothelial cells. Capillaries were counted in a definite area at ×200 magnification in each tumor. Vascular density was defined as the number of vessels per 100 areas.

Statistics

Correlation between vascular density and Epo content was evaluated by Spearman nonparametric rank correlation, and difference in Epo content between a tumor and its surrounding cirrhotic tissues was evaluated by paired *t*-test. Different in Epo content according to the histologic type of tumor was evaluated by non-paired *t*-test. A *P* value of less than 0.05 was considered to be significant.

Results

Of the 30 tumors, 13 were HCCs; 8, cholangiocell carcinomas; 7, poorly differentiated carcinomas; and 2, hamartomas.

The Epo content ranged from 6.1 to 97.8 mU/ml, with a median of 21.8 mU/ml in all 30 tumors, and Epo content was less than 5.0 mU/ml in the normal liver tissues and cirrhotic tissues without hepatic tumors. HCCs contained 22 ± 11 mU/ml Epo (mean ± SD); cholangiocell carcinomas, 43 ± 22 mU/ml; poorly differentiated carcinomas, 34 ± 20 mU/ml; and hamartomas, 10 ± 4 mU/ml. There was no significant difference in the Epo content according to the histologic type of tumors.

The vascular density values per 100 areas were 263 ± 151 (mean ± SD) in HCCs, 205 ± 142 in cholangiocell carcinomas, 210 ± 124 in poorly differentiated carcinomas, and 114 ± 22 in hamartomas (Table 1). There was a significant correlation between Epo content and vascular density in the analysis of all 30 tumors (Fig. 1; correlation coefficient, 0.480; $P = 0.01$). A significant correlation was also noted for the 13 HCCs (Fig. 2; correlation coefficient, 0.671; $P = 0.02$). However, there was no significant correlation for the

Table 1. Content of erythropoietin (Epo) and vascular density in hepatic tumors

Type of tumor	Content of Epo (mU/ml)	Vascular density per 100 areas
HCC (n = 13)	22 ± 11 (9–33)	263 ± 151
Cholangiocell ca. (n = 8)	43 ± 22 (21–65)	205 ± 142
Poorly diff. ca. (n = 7)	34 ± 20 (14–54)	210 ± 124
Hamartoma (n = 2)	10 ± 4 (6–14)	114 ± 22

Values are means ± SD, with ranges in parentheses
HCC, hepatocellular carcinoma; diff. differentiated; ca., carcinoma

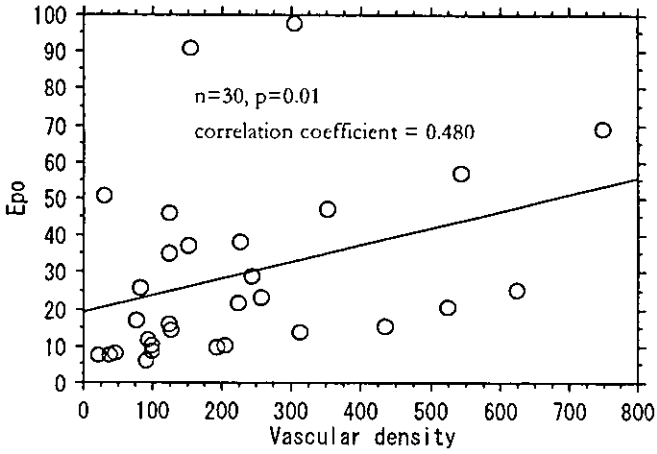


Fig. 1. Correlation between erythropoietin (*Epo*) content and vascular density for all 30 hepatic tumors. There was a significant correlation by Spearman nonparametric rank correlation (correlation coefficient, 0.480; $P = 0.01$). A linear regression line is shown

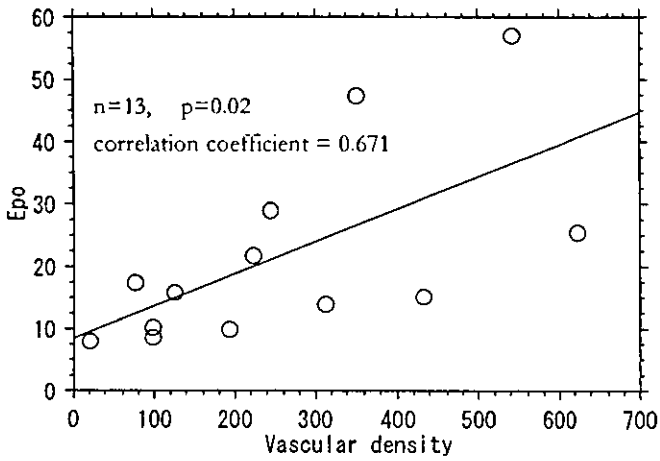
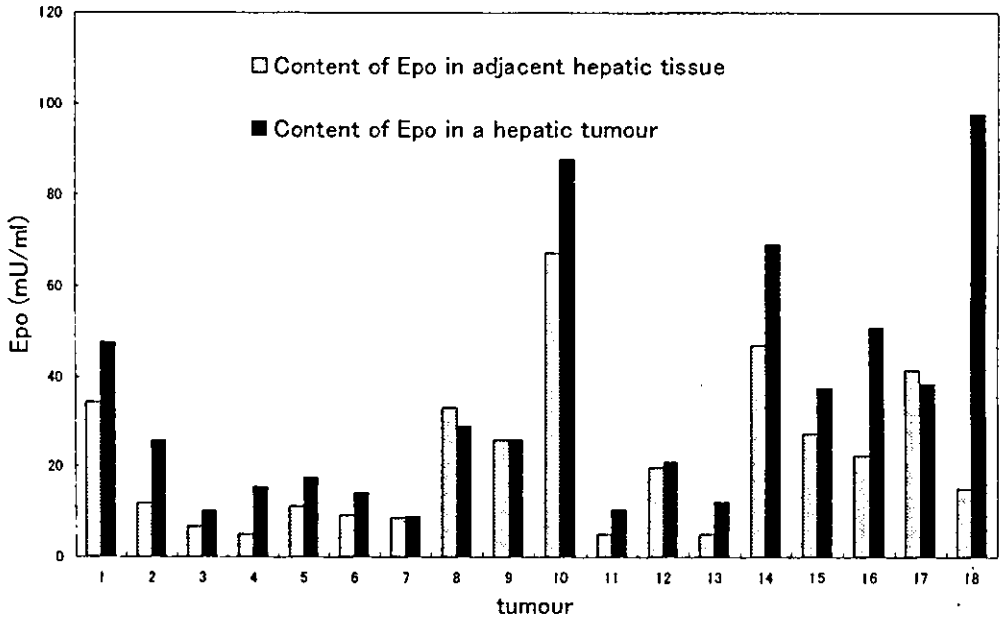


Fig. 2. Correlation between Epo and vascular density for 13 hepatocellular carcinomas (HCCs). A significant correlation between Epo and vascular density was noted by Spearman nonparametric rank correlation (correlation coefficient, 0.671; $P = 0.02$). A linear regression line is shown

Fig. 3. The content of Epo in 16 of the 18 hepatic tumors (excepting 2 tumors; nos 8 and 17) was equal to or higher than that in the adjacent cirrhotic tissues obtained 3–7 mm from the edge of each tumor. The mean Epo content of the 18 tumors was significantly higher than that of the adjacent cirrhotic tissues ($P < 0.05$; paired *t*-test)



eight cholangiocell carcinomas. In addition, no significant correlation was noted between tumor size and Epo content or between tumor size and vascular density.

For all rats fed 3'-Me-DAB for 5 months, the whole of the hepatic tissue showed cirrhotic changes macroscopically. Figure 3 shows the relation between the content of Epo in 18 tumors and the cirrhotic tissues adjacent to the tumors. In 16 of the 18 tumors, the Epo content was higher than that of adjacent cirrhotic tissues. The mean Epo content of the tumors was significantly higher than that of the adjacent hepatic tissues ($P < 0.05$; paired *t*-test).

Figure 4 shows the immunohistochemical staining for Epo-R. For all 30 tumors, Epo-R was detected in the endothelium of the intervening vessels. On the other hand, no immunoreactive Epo-R was discernible in the wall of the hepatic vasculature in the cirrhotic tissues adjacent to the tumors.

Discussion

In the present study, content of Epo and localization of Epo-R were investigated using chemically induced rat hepatic tumors. In this study, Epo was significantly more common in all the hepatic tumors, including HCCs, cholangiocell carcinomas, poorly differentiated carcinomas, and hamartomas, than in normal liver tissues and cirrhotic tissues. In addition, the Epo content was significantly higher

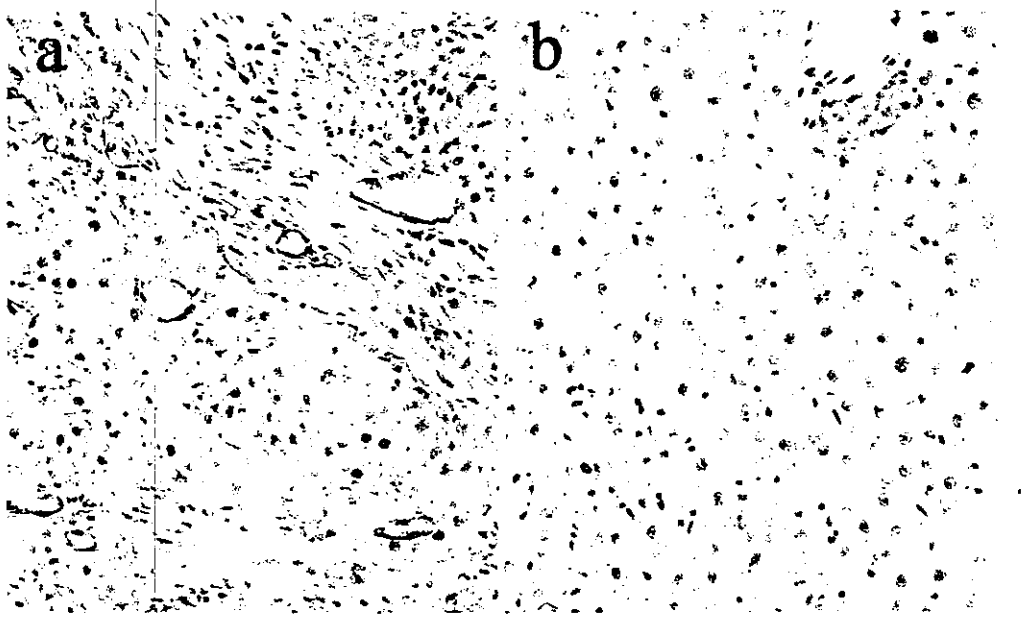


Fig. 4a,b. Immunohistochemical staining for Epo-R. Epo-R was detected in the wall of the intervening vessels in HCC, although no staining was noted in the wall of bile ducts (**a**). No immunoreactive Epo-R was discernible in the wall of the hepatic vasculature in the cirrhotic tissues adjacent to the tumors (**b**). **a**, **b** ×200

in tumor tissues than in the adjacent cirrhotic tissues (Fig. 3).

These results suggest that the murine hepatic tumor cells produce Epo. Although expression of Epo mRNA was not evaluated in the present study, Epo mRNA is expressed in HepG2, which is a human hepatoma cell line.[8] In addition to the reports of other human hepatoma cell lines that produce Epo,[18] there are clinical case reports of Epo-producing HCCs with erythrocytosis.[15,16] Erythrocytosis is one of the common paraneoplastic syndromes in HCC, with an incidence ranging from 2% to 12%.[16] As Epo is normally produced by the fetal liver, the production of Epo by hepatic tumors may represent a dedifferentiated function of the tumor cells. In this study, the presence of erythrocytosis in rats with tumors showing a high Epo content was not demonstrated, as the number of red blood cells was not counted. However, erythrocytosis is unlikely to have occurred in the rats, because their tumors were very small in diameter. In the clinical reports, erythrocytosis was reported in patients with advanced HCCs.[15,16]

There is accumulated evidence that Epo has angiogenic activity.[3] Epo stimulates the proliferation and migration of human and bovine endothelial cells, and it also stimulates angiogenesis in the rat thoracic aorta and mouse cyclic uterine angiogenesis.[6,12] Epo is involved not only in physiological angiogenesis but also in tumor angiogenesis of ovarian and uterine cancers.[8] In the present study, a significant correlation between Epo content and tumor vascular density was demonstrated for hepatic tumors (Figs. 1, 2). In addition, immunohistochemical staining revealed the presence of Epo-R in the endothelium of intervening tumor vessels. These findings suggest the presence of a loop in the Epo/Epo-R system, i.e., Epo is secreted by hepatic tumor cells and it affects vascular edothelial cells via its receptors, and promotes angiogenesis in a paracrine manner. It is, thus, suggested that Epo is an important factor for the angiogenesis of murine hepatic tumors.

In the present analysis, no immunoreactive Epo-R was detected in the wall of the hepatic vasculature in the cirrhotic tissues adjacent to the tumors, although Epo-R was noted in the endothelium of intervening tumor vessels. The reason for this selective expression of Epo-R in the tumor vessels is unclear. The immature nature of tumor vessels compared with mature hepatic vessels may be related to the selective expression of Epo-R.

HCC is a distinctively hypervascular tumor and angiogenesis is important in the process of hepatocarcinogenesis. Portal blood supply is gradually replaced by arterial blood supply with the progression of HCC. Vascular endothelial growth factor (VEGF) is known as one of the most potent angiogenic factors, and its role in the angiogenesis and development of HCC has been reported.[19,20] Because hypoxia increases the production of both Epo and VEGF, both systems may act in the angiogenesis mechanism of hepatic tumors.[1,2] In the present study, unfortunately, the content of VEGF could not be measured because of the small volume of the tumor tissues. In addition, immunostaining for VEGF failed in this study, due to unknown reasons. In a future study, the relationship between Epo and VEGF could be evaluated. Such a study would be helpful to understand angiogenesis in HCC.

As we have successfully demonstrated that inhibition of Epo signalling, by the injection of anti-Epo monoclonal antibody or a soluble form of Epo-R, resulted in tumor growth delay of ovarian and uterine cancers,[8] the present results may have implications for the treatment of hepatic cancers by inhibition of the Epo/Epo-R system.

Acknowledgments This study was supported in part by a Grant-in-Aid for Scientific Researh (13218128) from the Ministry of Education, Science, Sports, and Culture, Japan.

References

1. Krantz SB (1991) Erythropoietin. Blood 77:419–434
2. Marti HH, Wenger RH, Rivas LA, et al. (1996) Erythropoietin gene expression in human, monkey and murine brain. Eur J Neurosc 8:666–676
3. Masuda S, Nagao M, Sasaki R (1999) Erythropoietic, neurotrophic, and angiogenic functions of erythropoietin and regulation of erythropoietin production. Int J Hematol 70:1–6
4. Yasuda Y, Nagao M, Okano M, et al. (1993) Localization of erythropoietin and erythropoietin-receptor in postimplantation mouse embryos. Dev Growth Differ 35:711–722
5. Yasuda Y, Okano M, Nagao M, et al. (1996) Eythropoietin in mouse avascular yolk sacs is increased by retinoic acid. Dev Dyn 207:184–194
6. Yasuda Y, Masuda S, Chikuma M, et al. (1998) Estrogen-dependent production of erythropoietin in uterus and its implication in uterine angiogenesis. J Biol Chem 273:25381–25387
7. Yasuda Y, Fujita Y, Musya T, et al. (2001) Expression of erythropoietin in human female reproductive organs. Ital J Anat Embryol 106:215–222
8. Yasuda Y, Musya T, Tanaka H, et al. (2001) Inhibition of erythropoietin signalling destroys xenografts of ovarian and uterine cancers in nude mice. Br J Cancer 84:836–843
9. Yasuda Y, Fujita Y, Masuda S, et al. (2002) Erythyropoietin is involved in growth and angiogenesis in malignant tumours of female reproductive organs. Carcinogensis 23:1797–1805
10. Anagnostou A, Liu Z, Steiner M, et al. (1994) Erythropoietin receptor mRNA expression in human endothelial cells. Proc Natl Acad Sci USA 91:3974–3978
11. Yamaji R, Okada T, Moriya M, et al. (1996) Brain capillary endothelial cells express two forms of erythropoietin receptor mRNA. Eur J Biochem 239:494–500
12. Anagnostou A, Lee ES, Kessimian N, et al. (1990) Erythropoietin has a mitogenic and positive chemotactic effect on endothelial cells. Proc Natl Acad Sci USA 87:5978–5982
13. Carlini RG, Reyes AA, Rothstein M (1995) Recombinant human erythropoietin stimulates angiogenesis in vitro. Kidney Int 47:740–745
14. Ribatti D, Presta M, Vacca A, et al. (1999) Human erythropoietin induces a pro angiogenic phenotype in cultured endothelial cells and stimulates neovascularization in vivo. Blood 93:2627–2639
15. Okabe T, Urabe A, Kato T, et al. (1985) Production of erythropoietin-like activity by human renal and hepatic carcinomas in cell culture. Cancer 55:1918–1923
16. Matsuyama M, Yamazaki O, Horii K, et al. (2000) Erythrocytosis caused by an erythropoietin-producing hepatocellular carcinoma. J Surg Oncol 75:197–202
17. Kawamori Y, Matsui O, Kadoya M, et al. (1992) Differentiation of hepatocellular carcinomas from hyperplastic nodules induced in rat liver with ferrite-enhanced MR imaging. Radiology 183:65–72
18. Goldberg MA, Glass GA, Cunningham JM, et al. (1987) The regulated expression of erythropoietin by two human hepatoma cell lines. Proc Natl Acad Sci USA 84:7972–7976
19. Park YN, Kim YB, Yang KM, et al. (2000) Increased expression of vascular endothelial growth factor and angiogenesis in early stage of multistep hepatocarcinogenesis. Arch Pathol Lab Med 124:1061–1065
20. Yamaguchi R, Yano H, Nakashima Y, et al. (2000) Expression and localization of vascular endothelial growth factor receptors in human hepatocellular carcinoma and non-HCC tissues. Oncol Rep 7:725–729

An increase in the percentage of HLA-DR-positive peripheral leukocytes predicts a poor prognosis in patients with squamous cell carcinoma of the lung

HARUHIKO NAKAMURA, AUTE IDIRIS, YASUFUMI KATO and HARUBUMI KATO

Department of Surgery, Tokyo Medical University Hospital, 6-7-1 Nishi-shinjuku, Shinjuku-ku, Tokyo 160-0023, Japan

Abstract. Immunologic factors that predict survival in patients with lung cancer have not been established. We examined the relationship between the percentage of HLA-DR-positive peripheral leukocytes [HLA-DR$^+$ (%)] and survival of patients with squamous cell carcinoma of the lung. Before initiating therapy, peripheral blood was taken from 105 patients with squamous cell carcinoma of the lung. HLA-DR positivity was determined by flow cytometry. Patients were divided into 2 groups; a high and a low percentage group. The significance of the intergroup difference in the Kaplan-Meier survival curves was determined by the log rank test. Multivariate analysis was performed using the Cox proportional hazards model. The average HLA-DR$^+$ (%) was 25.9±10.6% (mean ± SD). Survival in the high percentage group (HLA-DR$^+$ (%) ≥25.9%, n=44) was much worse than that in the low percentage group (HLA-DR$^+$ (%) <25.9%, n=61; p=0.0002). The 5-year survival rate in the high percentage group was only 7.4%, while that in the low group was 54.3%. Multivariate analysis identified a significant association between survival and lymph node metastasis (p=0.0028) and HLA-DR$^+$ (%) (p=0.0004). Survival of patients with stages I, II, and IIIA was worse in the high percentage group (n=32) than that in the low percentage group (n=43; p<0.0001). However, survival of patients with more advanced disease, stages IIIB and IV, was similar in the high percentage (n=12) and low percentage groups (n=18; p=0.7610). The peripheral HLA-DR$^+$ (%) predicts survival of patients with resectable squamous cell carcinoma of the lung.

Introduction

Immunologic interactions between host and tumor are an important determinant of survival in cancer patients. However, the immunologic factors that predict survival in lung cancer have not been well characterized. We previously investigated several cell surface markers, NK activity, and lymphoblastogenesis in peripheral blood leukocytes taken from lung cancer patients looking for factors that correlate with a prognosis (1,2). Although other investigators have reported some of these cellular immunologic factors predicted survival (3-7), we found that the percentage of HLA-DR-positive peripheral leukocytes [HLA-DR$^+$ (%)] was the most reliable prognostic factor in lung cancer. The present study analyzed clinical data from 105 patients with squamous cell carcinoma of the lung during a longer follow-up period than the previous study to determine whether or not HLA-DR$^+$ (%) predicts survival.

Patients and methods

One hundred and five patients with squamous cell carcinoma of the lung, treated from April 1995 to November 1998 in our institute, were enrolled in this study. These 98 men and 7 women had an average age of 67.7 years (range, 40-90 years). The diagnosis of squamous cell carcinoma was based upon cytologic or histologic examinations. American Joint Committee on Cancer criteria were used for TNM staging of lung cancer (8). Stages IA and IB patients were grouped together as stage I, and stages IIA and IIB patients were grouped as stage II. Pathologic staging was used when resection was performed, and clinical staging when it was not. When surgery was not performed, the presence of lymph node metastasis was determined by chest computed tomography (CT). The presence of distant metastases was determined by brain CT, chest CT, abdominal CT, and bone scintigraphy. Thirty-nine patients had stage I disease, 10 had stage II, 26 had stage IIIA, 24 had stage IIIB, and 6 had stage IV. Fifty-eight patients underwent surgery and 47 patients did not undergo surgery. Photodynamic therapy (9) was performed for centrally located early squamous cell carcinoma. The mean follow-up period for patients alive was 45.6 months.

Before initiation of therapy, peripheral blood samples were obtained. The HLA-DR$^+$ (%) was determined as described previously (1). In short, the HLA-DR$^+$ cells were stained using a lysed whole blood method: 50 ml of blood from each patient was stained with 50 ml of diluted fluorescein isothiocyanate (FITC)-labeled anti-HLA-DR monoclonal antibody, Leu HLA-DR (Becton-Dickinson, Franklin Lakes, NJ), for 30 min at 4˚C in the dark. Then the red blood cells were lysed using buffer. After 15 min, the samples were analyzed by flow cytometry using the FCM-1 (Nihon-bunko, Tokyo,

Correspondence to: Dr Haruhiko Nakamura, Department of Surgery, Tokyo Medical University Hospital, 6-7-1 Nishi-shinjuku, Shinjuku-ku, Tokyo 160-0023, Japan
E-mail: hanakamu@tokyo-med.ac.jp

Key words: tumor immunity, lung cancer, squamous cell carcinoma, HLA-DR, leukocyte, survival

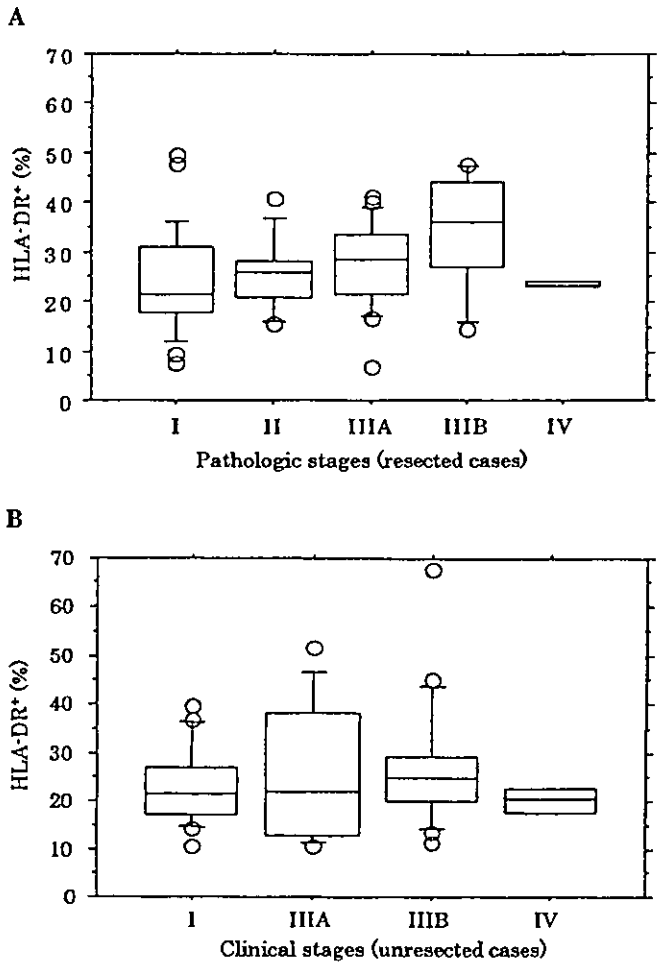


Figure 1. A, Comparison of the percentages of HLA-DR-positive peripheral leukocytes as a function of the pathologic stage in patients who underwent resection of squamous cell carcinoma of the lung. Differences between stages were not significant by the Kruskal-Wallis test (p=0.2976). B, Comparison of the percentages of HLA-DR-positive peripheral leukocytes as a function of the clinical stage in patients who did not undergo resection of squamous cell carcinoma of the lung. Differences between stages were not significant by the Kruskal-Wallis test (p=0.5406).

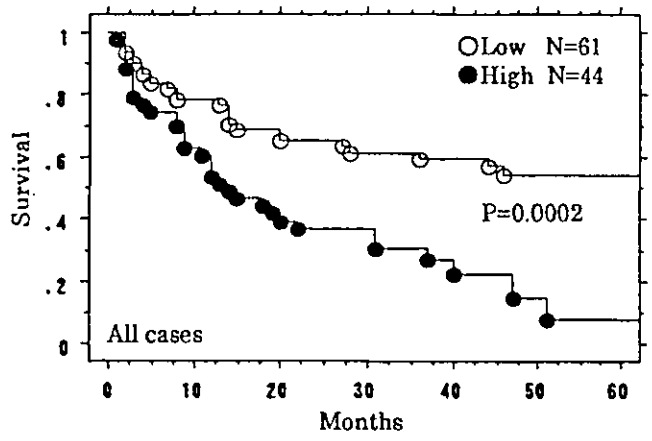


Figure 2. Kaplan-Meier overall survival curves comparing the HLA-DR-positive peripheral leukocytes high percentage group and the low percentage group in 105 patients. The difference was significant by the log rank test (p=0.0002).

Table I. Survival analysis of 105 patients with squamous cell carcinoma of the lung in a Cox proportional hazards model.

Prognostic factor	Multivariate analysis P-value (HR: 95% CI)
Age	
≥67.7 vs. <67.7	0.3934 (1.015: 0.981-1.051)
Gender	
Men vs. women	0.1399 (4.622: 0.606-35.10)
T-factor	
T_{2-4} vs. T_1	0.2555 (1.499: 0.746-3.012)
N-factor	
N_{1-3} vs. N_0	0.0028 (2.728: 1.414-5.263)
M-factor	
M_1 vs. M_0	0.2925 (1.715: 0.628-4.695)
HLA-DR⁺ (%)	
≥25.9 vs. <25.9	0.0004 (2.743: 1.565-4.808)

HR, hazard ratio; CI, confidence interval.

Japan). Results are expressed as percentages of the total leukocyte count.

The significance of differences in the HLA-DR⁺ (%) between groups were compared using the Mann-Whitney U test for 2 groups and the Kruskal-Wallis test for ≥3 groups. The patients were divided into a high percentage group, greater than or equal to the average percentage and a low percentage group, less than the average, to determine the correlation between HLA-DR⁺ (%) and survival. The survival rate was calculated by the Kaplan-Meier method. Survival differences were compared using the log rank test. A multivariate survival analysis was evaluated according to the Cox proportional hazards model in order to detect independent risk factors adjusting for the confounding factors. P<0.05 was considered significant.

Results

The average HLA-DR⁺ (%) in all 105 patients was 25.9±10.6% (mean ± SD). The HLA-DR⁺ (%) was similar in patients who did and did not undergo resection (p=0.3179). Additionally, HLA-DR⁺ (%) between stages was similar overall and in patients who did and did not undergo resection (Fig. 1).

Survival in the high percentage group [HLA-DR⁺ (%) ≥25.9 %; n=44] was significantly worse than in the low percentage group [HLA-DR⁺ (%) <25.9%; n=61] with 5-year survival rates of 7.4 and 54.3%, respectively (Fig. 2). Multivariate analysis including age, gender, T-factor, N-factor, M-factor, and HLA-DR⁺ (%) as co-variables indicated a significant association between survival and lymph node metastasis (p=0.0028) and HLA-DR⁺ (%) (p=0.0004) (Table I).

Among the 58 patients who underwent resection, survival in the high percentage group (n=29) was worse than that in the low group (n=29), with 5-year survival rates of 9.3 and 67.6%, respectively (Fig. 3). Among the 47 patients who did

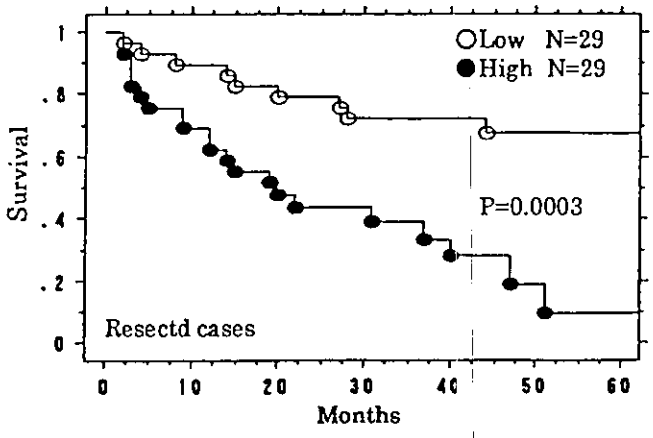


Figure 3. Kaplan-Meier survival curves comparing the HLA-DR-positive peripheral leukocytes in high and low percentage group in 58 patients who underwent resection of squamous cell carcinoma of the lung. The difference was significant by the log rank test (p=0.0003).

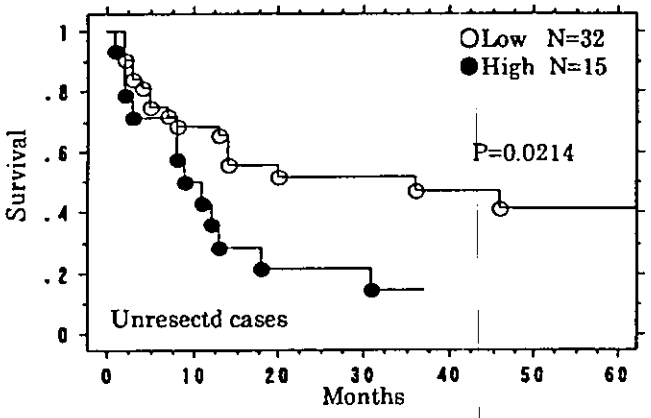


Figure 4. Kaplan-Meier survival curves comparing the HLA-DR-positive peripheral leukocytes in high and low percentage group in 47 patients who did not undergo resection of squamous cell carcinoma of the lung. The difference was significant by the log rank test (p=0.0214).

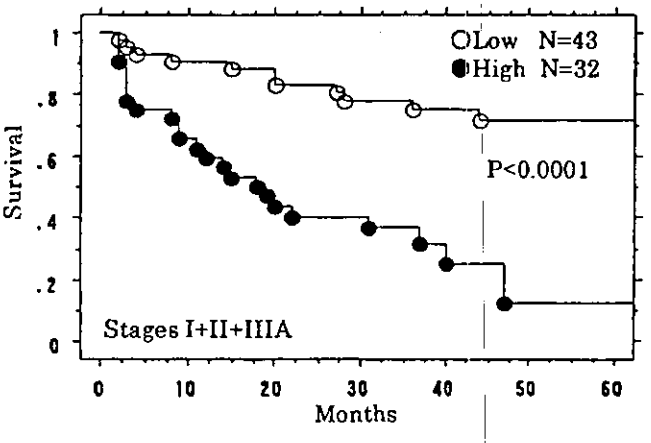


Figure 5. Kaplan-Meier survival curves comparing the HLA-DR-positive peripheral leukocytes in high and low percentage group in stages I, II, and IIIA of patients with squamous cell carcinoma of the lung. The difference was significant by the log rank test (p<0.0001).

not undergo resection, survival in the high percentage group (n=15) was worse than in the low percentage group (n=32), with no 5-year survivors in the high percentage group and a 41.5% survival rate in the low percentage group (Fig. 4).

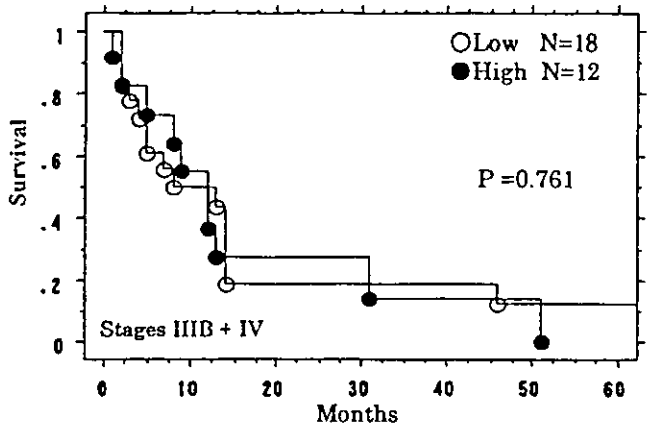


Figure 6. Kaplan-Meier survival curves comparing the HLA-DR-positive peripheral leukocytes in high and low percentage group in stages IIIB and IV of patients with squamous cell carcinoma of the lung. The difference was not significant by the log rank test (p=0.7610).

Among patients with stages I, II, or IIIA disease, survival in the high percentage group (n=32) also was worse than in the low percentage group (n=43), with 5-year survival rates of 12.5 and 71.5%, respectively (Fig. 5). However, survival in the high (n=12) and the low (n=18) percentage groups were similar in patients with more advanced disease, stages IIIA and IV (Fig. 6).

Discussion

HLA-DR antigens are shared by activated T cells, activated NK cells, B cells, monocytes, macrophages, dendritic cells, and hematopoietic progenitor cells (10). A decrease in peripheral blood monocyte HLA-DR expression has been correlated with a poor prognosis in patients with severe injury or sepsis (11-13). Conversely, our study of patients with lung cancer revealed that an increase in HLA-DR$^+$ (%) strongly correlates with a poor outcome. We performed additional flow cytometric analyses to determine the HLA-DR$^+$ subset more precisely using double-staining with combinations of HLA-DR/CD4 and HLA-DR/CD8 in another series of patients with lung cancer. In that study, the subset that best predicted poor survival was HLA-DR$^+$ CD8$^-$ leukocytes (14).

We believe the following facts are relevant: i) HLA-DR$^+$ (%) does not increase with stage progression. Since the HLA-DR$^+$ (%) does not rise to maintain a correlation with the pathologic or clinical stages, some immunologic reactions probably are induced by only some tumor cells through an increase in the number of HLA-DR$^+$ cells. ii) HLA-DR$^+$ (%) predicts survival in patients with squamous cell carcinoma, but not in patients with adenocarcinoma (2). This finding implies that tumor immunogenicity plays an important role in determining survival. For example, spontaneous regression of squamous cell carcinoma of the lung has been the subject of several reports (15-19), but this phenomenon has not been documented in adenocarcinoma of the lung, probably due to lower immunogenicity of this histologic type. iii) Survival differences related to the HLA-DR$^+$ (%) disappear in stages IIIB and IV. This may be because the host immune response is weakened in advanced disease.

The status of HLA-DR expression by peripheral leukocytes and prognosis in cancer patients has received little attention. Tisch *et al* (20) studied peripheral leukocytes in patients with head and neck squamous cell carcinoma and found a negative correlation between survival and HLA-DR6 positivity. Yacyshyn *et al* (21) reported that patients with breast cancer who showed a greater than median decrease in peripheral $CD20^{-}HLA\text{-}DR^{+}$ cells following cyclophosphamide treatment had a survival advantage over patients who had less than the median decrease in the percent of the same subset. Kikuchi *et al* (22) reported that the percentage of peripheral $CD3^{+}HLA\text{-}DR^{+}$ cells in patients with ovarian cancer with minimal residual tumors after surgery was higher than it was pre-operatively, while the value in patients with a large residual tumor volume was lower. Arista *et al* (23) reported greater numbers of peripheral $HLA\text{-}DR^{+}$ T lymphocytes in patients with colorectal cancer than in healthy volunteers. These reports on breast, ovarian, and colorectal cancer suggested that certain subsets of $HLA\text{-}DR^{+}$ cells are induced by the presence of cancer cells. We do not believe that the stimulation of leukocytes by cancer cells is always advantageous in cancer patients. Although we did not analyze cytokines in the present study, a Th1/Th2 imbalance in cancer patients (24-26) may play an important role in killing cancer cells in the host's immune response. Ito *et al* (27) reported that the Th1/Th2 ratio in infiltrative lymphocytes is elevated in patients with squamous cell carcinoma of the lung. In addition, Gerrard *et al* (28) reported that a Th2-type cytokine, interleukin 4, increases HLA-DR expression in normal human monocytes. Thus, an increase in the $HLA\text{-}DR^{+}$ (%) in patients may reflect a Th2-dominant state that makes the prognosis in these patients worse. Further studies are needed on this point.

In conclusion, $HLA\text{-}DR^{+}$ (%) can be a useful immunologic marker to predict survival in potentially respectable, stages IA to IIIA squamous cell carcinoma of the lung.

References

1. Nakamura H, Kawasaki N, Hagiwara M, Saito M, Konaka C and Kato H: Cellular immunologic parameters related to age, gender, and stage in lung cancer patients. Lung Cancer 28: 139-145, 2000.
2. Nakamura H, Saji H, Ogata A, *et al*: Immunologic parameters as significant prognostic factors in lung cancer. Lung Cancer 37: 161-169, 2002.
3. Wanebo H, Rao B, Miyazawa N, *et al*: Immune reactivity in primary carcinoma of the lung and its relation to prognosis. J Thorac Cardiov Sur 72: 339-350, 1976.
4. Dalbow M, Concannon J, Eng J, Weil C, Conway J and Narayanan NP: Lymphocyte mitogen stimulation studies for patients with lung cancer: evaluation of prognostic significance of preirradiation therapy studies. J Lab Clin Med 90: 295-302, 1977.
5. Cannon G, Dean J, Herberman R, *et al*: Association of depressed postoperative lymphoproliferative responses to alloantigens with poor prognosis in patients with stage I lung cancer. Int J Cancer 25: 9-17, 1980.
6. Braun D, Nisius S, Hollinshead A and Harris J: Serial immune testing in surgically resected lung cancer patients. Cancer Immunol Immunother 15: 114-120, 1983.
7. Lin C, Kuo Y, Huang W and Lin C: Natural killer cell activity in lung cancer patients. Chest 92: 1022-1024, 1987.
8. Mountain C: Revisions in the International System for Staging Lung Cancer. Chest 111: 1710-1717, 1997.
9. Nakamura H, Kawasaki N, Hagiwara M, Ogata A and Kato H: Endoscopic evaluation of centrally located early squamous cell carcinoma of the lung. Cancer 91: 1142-1147, 2001.
10. Cheadle W: The human leukocyte antigens and their relationship to infection. Am J Surg 165: S75-S81, 1993.
11. Hershman M, Cheadle W, Wellhausen S, Davidson P and Polk H: Monocyte HLA-DR antigen expression characterizes clinical outcome in the trauma patient. Br J Surg 77: 204-207, 1990.
12. Cheadle W, Hershman M, Wellhausen S and Polk H: HLA-DR antigen expression on peripheral blood monocytes correlates with surgical infection. Am J Surg 161: 639-645, 1991.
13. Ditschkowski M, Kreuzfelder E, Rebmann V, *et al*: HLA-DR expression and soluble HLA-DR levels in septic patients after trauma. Ann Surg 229: 246-254, 1999.
14. Nakamura H, Saji H, Idiris A, *et al*: Peripheral leukocytes with $HLA\text{-}DR^{+}/CD8^{-}$ phenotype is associated with a prognosis in patients with lung cancer. Anticancer Res (In press).
15. Cole W: Relationship of causative factors in spontaneous regression of cancer to immunologic factors possibly effective in cancer. J Surg Oncol 8: 391-411, 1976.
16. Blades B and McCorkle R: A case of spontaneous regression of an untreated bronchogenic carcinoma. J Thorac Surg 27: 415-419, 1954.
17. Bell J, Jesseph J and Leighton R: Spontaneous regression of bronchogenic carcinoma with five year survival. J Thorac Cardiov Sur 48: 984-990, 1964.
18. Smith A: Cure of lung cancer from incomplete surgical resection. BMJ 5: 563-565, 1971.
19. Sperduto P, Vaezy A, Bridgman A and Wilkie L: Spontaneous regression of squamous cell lung carcinoma with adrenal metastasis. Chest 94: 887-889, 1988.
20. Tisch M, Kyrberg H, Weidauer H, *et al*: Human leukocyte antigens and prognosis in patients with head and neck cancer: results of a prospective follow-up study. Laryngoscope 112: 651-657, 2002.
21. Yacyshyn M, Poppema S, Berg A, *et al*: $CD69^{+}$ and $HLA\text{-}DR^{+}$ activation antigens on peripheral blood lymphocyte populations in metastatic breast and ovarian cancer patients: correlations with survival following active specific immunotherapy. Int J Cancer 61: 470-474, 1995.
22. Kikuchi Y, Iwano I, Kita T, *et al*: Changes of lymphocyte subsets in peripheral blood before and after operation of patients with advanced ovarian carcinoma. J Cancer Res Clin Oncol 116: 283-287, 1990.
23. Arista M, Callopoli A, De Franceschi L, *et al*: Flow cytometric study of lymphocyte subsets in patients at different stages of colorectal carcinoma. Dis Colon Rectum 37: S30-S34, 1994.
24. Yamamura M, Modlin R, Ohmen J and Moy R: Local expression of antiinflammatory cytokines in cancer. J Clin Invest 91: 1005-1010, 1993.
25. Pellegrini P, Berghella A, Del Beato T, Cicia S, Adorno D and Casciani C: Disregulation in TH1 and TH2 subsets of $CD4^{+}$ T cells in peripheral blood of colorectal cancer patients and involvement in cancer establishment and progression. Cancer Immunol Immunother 42: 1-8, 1996.
26. Huang M, Sharma S, Mao J and Dubinett S: Non-small cell lung cancer-derived soluble mediators and prostaglandin E2 enhance peripheral blood lymphocyte IL-10 transcription and protein production. J Immunol 157: 5512-5520, 1996.
27. Ito N, Nakamura H, Tanaka Y and Ohgi S: Lung carcinoma: analysis of T helper type 1 and 2 cells and T cytotoxic type 1 and 2 cells by intracellular cytokine detection with flow cytometry. Cancer 85: 2359-2367, 1999.
28. Gerrard T, Dyer D and Mostowski H: IL-4 and granulocyte-macrophage colony-stimulating factor selectively increase HLA-DR and HLA-DP antigens but not HLA-DQ antigens on human monocytes. J Immunol 144: 4670-4674, 1990.

Outcome of Surgery for Small Cell Lung Cancer – Response to Induction Chemotherapy Predicts Survival

H. Nakamura[1, 2]
Y. Kato[2]
H. Kato[2]

Abstract

Background: The role of surgery for local control of small cell lung cancer (SCLC) is controversial. **Methods:** Sixty-nine consecutive patients who underwent complete resection of SCLC in our hospital were reviewed. The patients included 62 men and 7 women. Clinical stage at the time of diagnosis was c-stages IA and B in 29, c-stages IIA and B in 12, c-stage IIIA in 21, and c-stage IIIB in 7. **Results:** Thirty-two patients received induction chemotherapy, and 37 patients underwent initial surgery. The overall response rate to induction chemotherapy was 71.9%. The survival rate stratified by clinical stage at the time of diagnosis was 48.9% for c-stage I, 33.3% for c-stage II, 20.2% for c-stage IIIA, and 0% for c-stage IIIB. Downstaging after induction chemotherapy conferred a survival benefit. Survival after lobectomy or bilobectomy was better than after pneumonectomy. Patients who received adjuvant chemotherapy survived longer than patients who did not. **Conclusions:** Surgery combined with chemotherapy is a therapeutic option in selected patients with SCLC. Pathologic nodal status and response to induction chemotherapy are predictors of survival.

Key words
Chemotherapy · lung cancer · surgery · survival · small cell lung cancer

Introduction

Small cell lung cancer (SCLC) is considered a systemic disease, because the potential for hematogenous and lymphogenic metastases is high. At present, concurrent chemoradiotherapy for limited disease (LD) and chemotherapy for extensive disease (ED) are standard practice. About 30 years ago, a randomized study by the British Medical Research Council [1] concluded that radiotherapy alone for LD was superior to surgery. However, the local recurrence rate after radiation therapy alone subsequently was reported to be 18% to 69% [2]. The Veteran's Administration Surgery Oncology Group [3] reviewed data on 148 resected SCLCs to evaluate the role of adjuvant chemotherapy in non-small cell lung cancers (NSCLCs) and reported a 59.5% 5-year survival rate for stage IA disease. Since then, several series looking at the role of surgery for SCLC have been reported from different institutions. The University of Toronto Lung Oncology Group [4] treated 119 SCLCs with surgery and multi-modality therapy. The overall 5-year survival rate in that study was 39%, and the rates stratified by pathologic stage were 51% in stage I, 28% in stage II, and 19% in stage III. These survival rates were relatively good and represent an acceptable outcome.

To define the role of surgery for SCLC, the Lung Cancer Study Group [5] randomized cases of LD excluding stage I, to undergo resection or not after 5 cycles of chemotherapy with CAV (cyclophosphamide [CPA] + adriamycin [ADR] + vincristine [VCR]) followed by radiation. In that study, surgery did not improve survival.

Affiliation
[1] Department of Respiratory Surgery, International University of Health and Welfare Atami Hospital, Shizuoka, Japan
[2] Department of Surgery, Tokyo Medical University Hospital, Tokyo, Japan

Correspondence
Haruhiko Nakamura, M.D., PhD · Department of Respiratory Surgery · International University of Health and Welfare Atami Hospital · 13-1 Higashikaigan-cho · Atami-City Shizuoka 413-0012 · Japan · Phone: +81557819171, Ext: 2906 · Fax: +81557836632 · E-mail: h.nakamura@iuhw.ac.jp

Received January 5, 2004

Bibliography
Thorac Cardiov Surg 2004; 52: 206–210 © Georg Thieme Verlag KG · Stuttgart · New York ·
DOI 10.1055/s-2004-821075 ·
ISSN 0171-6425

At present, the role of surgery combined with chemotherapy or radiotherapy for local control of SCLC is still controversial. Even when a radiographic complete response is obtained, up to 75% of patients have residual viable cancer cells in the surgical specimen [6]. Also, residual chemoresistant NSCLC coexist with SCLC in 10% to 25% of specimens resected after administration of chemotherapy [7]. Therefore we believe that complete resection of the primary tumor is indicated in some circumstances. In Germany, a phase II multicenter trial [8] to treat patients with advanced SCLC, stages IIB/IIIA, using combined modality therapy including surgery, proved effective in achieving local control and in increasing survival after complete resection. This is an encouraging outcome and validates the role of surgery for SCLC in combination with chemotherapy or radiotherapy. We retrospectively analyzed consecutive patients who underwent surgery for SCLC in our hospital to better define the role of surgery in this disease.

Patients and Methods

From January 1977 through December 2002, 79 patients underwent resection of an SCLC in our hospital. The 69 patients in whom complete resection was achieved were the subjects of this study. Table 1 shows the clinicopathologic characteristics of the study group. The patients included 62 men and 7 women, age range 39 to 79 years (mean, 62.2). Disease stage was determined based on the American Joint Committee on Cancer criteria [9]. Clinical stages at the time of diagnosis were c-stages IA and B in 29, c-stages IIA and B in 12, c-stage IIIA in 21, and c-stage IIIB in 7. Thirty-two patients received induction chemotherapy followed by surgery, and 37 patients underwent initial surgery. Forty-eight patients received adjuvant chemotherapy. In the induction chemotherapy group, 62.5% (20/32) patients had c-stage IIIA disease or higher stages. Conversely, only 22.6% (8/37) patients in the initial surgery group had c-stage IIIA disease or higher. Median follow-up of patients alive was 65 months.

The survival rate was calculated by the Kaplan-Meier method. Significance of the survival differences between groups was evaluated by the log rank test. A multivariate analysis was carried out according to the Cox proportional hazards model to identify independent risk factors. $p < 0.05$ was considered significant.

Results

Table 2 shows the therapy administered to the patients in this study. Most patients (59/69, 85.5%) received chemotherapy before and/or after surgery. We used CPA-based chemotherapy (CPA 800 mg/m^2 on day 1, ADR 50 mg/m^2 on day 1, and VCR 1.4 mg/m^2 on day 1) until the mid-1980s, and platinum-analog-based chemotherapy (cisplatin [CDDP] 80 mg/m^2 on day 1 and etoposide [VP-16] 100 mg/m^2 on day 1, 3 and 5, or carboplatin [CBDCA] 400 mg/m^2 and VP-16 100 mg/m^2 on day 1, 3 and 5) after the mid-1980s as the standard regimen. The numbers of cycles ranged from 1 to 6.

The overall radiographic response rate to induction chemotherapy was 71.9% (23/32): there was complete response in 4 (12.5%), partial response in 19 (59.4%), and stable disease in 9 (28.1%). Pathologic complete response was obtained in 3 cases (9.4%). The surgical specimens contained small cell carcinoma and another type of cancer, so-called combined small cell carcinoma [10], in 7.2% (5/69); combined small cell and adenocarcinomas were found in 3 and combined small cell and squamous cell carcinomas in 2 cases.

Table 1 Demographics and clinical characteristics of patients who underwent surgery for small cell lung cancer

	Total (n = 69)	*Induction chemotherapy (n = 32)*	*Initial surgery (n = 37)*
Gender			
Male	62	28	34
Female	7	4	3
Age			
Mean ± SD	62.2 ± 9.1	59.5 ± 7.8	64.5 ± 9.5
Clinical stage			
IA	15	1	14
IB	14	4	10
IIA	1	1	0
IIB	11	6	5
IIIA	21	15	6
IIIB	7	5	2
IV	0	0	0
Pathologic stage			
IA	21	9	2
IB	9	4	5
IIA	4	2	2
IIB	8	3	5
IIIA	16	9	7
IIIB	10	4	6
IV	1	1	0

Table 2 Combination chemotherapy regimens and surgery for small cell lung cancer

	Induction therapy (n = 32)		*Adjuvant therapy (n = 48)*[a]	
	Chemo.	*Chemo. + Rad.*	*Chemo.*	*Chemo. + Rad.*
CDDP or CBDCA based				
CDDP + VP-16	20	1	17	1
CBDCA + VP-16	3	1	13	0
CPA based				
CAV	5	2	11	6
Total	28	4	41	7

Chemo. = chemotherapy; Rad. = radiotherapy
CDDP = cisplatin; CBDCA = carboplatin; VP-16 = etoposide;
CPA = cyclophosphamide; CAV = CPA + ADR (adriamycin) + VCR (vincristine)
[a] Both induction and adjuvant therapy were performed in 21 patients.