1. Introduction

Transthoracic needle biopsy is a common procedure used mainly to elucidate the nature of pulmonary nodules [1,2]. CT has rapidly become the guidance modality of choice for performing transthoracic needle biopsy due to technical advances in CT and its better detection of pulmonary lesions, which sometimes cannot be identified on chest radiograph [3].

CT-guided needle biopsy is generally regarded as a safe procedure, although pneumothorax and other rare complications can sometimes occur [4]. There have been occasional reports of deaths due to severe complications, such as, air embolism following lung biopsy [5]. Fortunately, these complications are generally very rare; previously published data shows wide variations in complication rates, making them difficult to generalize [5–8].

The aim of our study was to update the rate of severe complications following CT-guided needle biopsy in Japan via a mailed survey.

2. Materials and methods

Postal questionnaires regarding CT-guided needle biopsy were sent out to named radiologists at 101 university hospitals and cancer centers in Japan in August 2001. The radiologists at these hospitals were asked to pass duplications of the questions to other associate hospitals. The questions required information regarding: the total number and duration of CT-guided lung biopsies performed at each hospital, and the complication rates, numbers of pneumothorax, hemothorax, air embolism, tumor seeding, tension pneumothorax, severe pulmonary hemorrhage or hemoptysis which was treated with drugs for hemostasis and other rare complications, and mortalities and morbidities after that.

We defined a case as having a severe complication when one of the following criteria was met: (1) the duration of hospital stay was prolonged due to the biopsy, (2) a special technique or treatment was required to treat the complication, (3) a special procedure was required for resuscitation, and (4) shock or pre-shock developed. Each severe complication was followed with additional questions, including diagnosis of the complication, the position of the pulmonary lesion, the distance of the pulmonary lesion from the peripheral pleura, whether the lesion was located near the hilum or large pulmonary vessel, whether there was any reasonable factor causing the complication such as cough during biopsy, biopsy technique (CT-fluoroscopy or Co-axial method), the number of biopsies for each case, type and size of the needle, and presence of significant sequela from the complication.

Furthermore, the questionnaire included the following enquiries: whether emergency medication was prepared for resuscitation in the operating room, whether the patient was treated by the intravenous route and monitors, such as automatic sphygmomanometer, pulse oximetry, and electrocardiography. Finally, availability of access to other departments in case of emergency was questioned. Postal replies of questionnaire had been received for a year, and these answers were analyzed.

3. Results

A total of 9783 biopsy data were collected from 124 centers. The average number of biopsies performed per center was 79 cases, and that per center per year was 21 cases. The number of institutions in which hyperbaric oxygen recompression can be performed was 41 of 114 (37%) hospitals. Patients were kept on peripheral intravenous drip infusion in 86 of 92 (93%) hospitals, automatic sphygmomanometer in 38 of 92 (41%) hospitals, pulse oximetry in 32 of 92 (35%) hospitals, and electrocardiography in 8 of 92 (9%) hospitals.

Pneumothorax was the most common complication, and occurred in 2412 (35%) of 6881 cases. The number of centers that reported severe complications was 39 (35%) of 114 centers. The total number of overall severe complications was 74 (0.75%) cases. Of these, details of the complications in 64 cases are described in Table 1. There were six cases (0.061%) with air embolism, six cases (0.061%) with tumor seeding at the site of the biopsy route, 10 cases (0.10%) with tension pneumothorax, six cases (0.061%) with severe pulmonary hemorrhage or hemoptysis, 10 cases (0.10%) with hemothorax, and 26 cases (0.26%) with others. The others included 14 cases of pneumothorax requiring temporal drainage of the pneumothorax or chest tube insertion, three cases of heart arrest, and so on. There was no report of coughing during needle placement into the thorax in any of the cases with air embolism. Two of six pulmonary lesions were complicated with air emboli located near the large pulmonary vessel, and one lesion contained a cavity (Table 2). Tumor seeding occurred in two cases following CT-guided biopsy performed

Table 1
Summary of 64 cases of severe complications

Severe complications	No.
Pneumothorax requiring drainage of air	14
Tension pneumothorax	10
Hemothorax	10
Air embolism	6
Tumor seeding	6
Pulmonary hemorrhage of hemoptysis	6
Heart arrest	3
Respiratory arrest	1
Shock	1
Cyanosis	1
Cardiac tamponade	1
Pneumomediastinum	1
Mediastinal hematoma	1
Loss of consciousness	1
Severe pain of biopsied site	1
disseminated intravascular coagulation (DIC)	1
Total	64

Table 2
Summary of cases of air embolism

No.	Age	Sex	Size (mm)	Location (lobe)	Distance from pleura (mm)	Large vessel near the nodule	Cavity	CT-fluoloscopy	Co-axial method	No. of biopsy	Technique of biopsy	Size of the needle	Sequela
1	72	F	20	Left lower	40	Yes	No	Yes	No	2	Core biopsy	18G	Death
2	59	M	10	Left lower	20	No	No	NA[a]	Yes	1	Core biopsy	18G	Totally improved
3	57	F	7	Right middle	25	No	No	Yes	No	1	Core biopsy	18G	Totally improved
4	74	M	20	Right upper	25	Yes	No	Yes	No	2	Core biopsy	20G	Partially improved
5	57	M	12	Right lower	3	No	No	No	Yes	1	Core biopsy	20G	Totally improved
6	75	M	25	Right lower	18	No	Yes	No	No	1	Core biopsy	18G	Totally improved

[a] NA, information was not available.

by the Co-axial method (Table 3). In one of these two cases, the tip of the outer cannula was placed within the chest wall, so that seeding obviously occurred by direct contact of the inner needle with the biopsy route.

From a total of 62 cases with severe complications, 54 cases (0.55%) were recovered without sequela, and one case (0.01%) recovered but with hemiplegia due to cerebral infarction. Unfortunately, four (0.04%) of the remaining seven cases died just after the CT-guided biopsy procedure; these consisted of one case of air embolism, one case of DIC, and two cases of heart arrest. Three cases (0.03%) of the remaining seven cases died several years later due to tumor seeding. Four cases complicated with air embolism, three of which were treated with hyperbaric oxygen recompression, were recovered without sequela out of a total of six cases. In 23 (50%) of 46 centers, an emergency team was able to attend when a severe complication occurred.

4. Discussion

Recently, many small pulmonary lesions, which cannot be detected on chest radiograph, have been easily visualized by CT examination in daily clinical work. These lesions are usually followed with CT, or in some cases these are biopsies using CT-guided technique. CT-guided needle biopsy is a widely accepted technique and is one of the principal methods for evaluating a pulmonary lesion [9]. Although it is not rare to have minor complications due to CT-guided needle biopsy, such as, a small amount of pneumothorax and pulmonary hemorrhage, these complications improve without any treatment [5]. On the other hand, it is well known that potentially life-threatening complications such as air embolism and tumor seeding can occur. Fortunately, the frequency of these complications is considered very rare [5]. However, the number of published reports has shown that the incidence of air embolism has been increasing over the last several years. Only seven cases with air embolism were documented in the 20 years before 1995 [10–16], whereas six cases have already been published in the last 10 years [17–22].

This is the first national research study demonstrating the incidence rate of severe complications with respect to CT-guided needle biopsy based on a large number of biopsy cases using a multi-center survey.

The most common complication of transthoracic percutaneous needle biopsy is pneumothorax, with a frequency rate of 0–61%, whereas the incidence of pneumothorax requiring chest tube drainage ranges from 1.6% to 17% [23]. In the present study, the rate of pneumothorax was 35.1%, which is considered comparable to the previous studies.

Sinner's review of the literature determined that there were two cases suspected of air embolism in 2726 patients [5]. He estimated that the relative risk of air embolism per patient was about 0.07%. In the present study of 9783 biopsies, air embolism occurred in six patients, resulting in an incidence

Table 3
Summary of cases of tumor seeding

No.	Age	Sex	Size (mm)	Location	Distance from pleura (mm)	Co-axial method	No. of biopsy	Technique of biopsy	Size of the needle
1	72	M	30	Right upper	0	No	1	Core biopsy	18G
2	73	M	30	Left lower	30	Yes	3	Core biopsy	18G
3	71	M	10	Right upper	20	No	2	Aspiration biopsy	22G
4	30	F	28	Left upper	76	No	2	Core biopsy	18G
5	69	M	15	Right lower	0	No	2	Core biopsy	21G
6	77	M	12	Right upper	30	Yes	2	Core biopsy	20G

rate of 0.06%, which also shows no major difference from the previously reported complication rate. However, in the present study, there were several cases of severe complications including cardiac and respiratory arrest, and shock, which can be secondary to air embolism, although it is very difficult to confirm air embolism in the coronary artery in cases of myocardial infarction when the patient has not been scanned at the level of the heart. It is speculated that concurrent cough during the procedure has a high possibility of an air embolism misplacing the biopsy needle into the large vessel adjacent to the pulmonary lesion. Among the total of six cases with air emboli in the present study, two cases demonstrated biopsied pulmonary lesions located close to the large vessels, however the remaining four cases have no close relation to the large vessels. There were no reports of coughing during the procedure in any of the cases complicated by air embolism. Air embolism even occurred in a case in which the nodule was very near the pleura (case no. 5). In our study, all cases with air emboli had undergone CT-guided biopsy using a core biopsy needle of 18–20 gauge, which is greater in diameter than the usually used fine aspiration needles. Having said that, in the previous reviews, most cases with air emboli were biopsied by fine aspiration needles, and there are two prior reports of air embolism following CT-guided lung needle marking using thin needles without recent biopsy [24–26].

Tumor seeding into the needle tract seems to be a rare possibility in several case reports [27–34]. There were six cases (0.06%) of tumor seeding in our study, which is a relatively high frequency compared to previous studies [5,35]. The true incidence of tumor seeding along the needle may be underestimated as not all cases can be diagnosed, and many patients die before these metastases become clinically apparent. Tumor seeding appears to depend on the size of the needle, therefore large-bore needles carry a relatively greater risk of tumor seeding, however tumor seeding following a fine needle aspiration was reported in one case of our study. It is thought that CT-guided biopsy performed using the Co-axial method has less frequency of tumor seeding as the outer cannula minimizes direct contact of the tumor cells with the biopsy route. Surprisingly, tumor seeding occurred in two cases using the Co-axial method. We speculate that the outer cannula was not appropriately placed.

Unfortunately, there were seven patients (0.07%) who died in our study due to complications in the CT-guided needle biopsy. Greene [6] estimated the mortality rate associated with fine needle aspiration to be 0.02%, however Richardson et al. [8] reported eight deaths (0.15%) in their study due to complications in CT-guided needle biopsy. Most of the deaths in the present study were attributed to fatal air embolism. Three cases of air embolism that were treated with hyperbaric oxygen recompression were recovered without sequela, which may suggest hyperbaric oxygen recompression therapy is effective for treatment of air embolism, and for reducing the mortality rate.

Our study has several limitations, including selection bias, the long period of the study, multi-center analysis with a large variety of techniques and CT scanners, and the possibility of missing or misdiagnosing significant complications such as the number of air emboli and tumor seeding. Moreover, our study is a retrospective questionnaire-based analysis rather than a prospective survey.

In conclusion, this is the first nation-wide study documenting severe complications with respect to CT-guided needle biopsy in Japan. The complication rate in Japan is comparable to internationally published figures. We believe this data will improve both clinicians as well as patients understanding of the risk versus benefit of CT-guided needle biopsy, resulting better decisions.

Acknowledgement

The authors, members of the Japanese lung biopsy conference, dedicate this manuscript to Dr. Junpei Ikezoe, originator of this conference. We are also grateful to those specialists who completed the questionnaire. The authors thank Dr. Javzandulam Natsag for his assistance with manuscript editing.

References

[1] Sinner WN. Pulmonary neoplasms diagnosed with transthoracic needle biopsy. Cancer 1979;43:1533–40.
[2] Klein JS, Zarka MA. Transthoracic needle biopsy. J Thorac Imag 1997;12:232–49.
[3] Hirose T, Mori K, Machida S, et al. Computed tomographic fluoroscopy-guided transthoracic needle biopsy for diagnosis of pulmonary nodules. Jpn J Clin Oncol 2000;30:259–62.
[4] Berquist TH, Bailey PB, Cortese DA, et al. Transthoracic needle biopsy: accuracy and complication in relation to location and type of lesion. Mayo Clin Proc 1980;55:475–81.
[5] Sinner WN. Complications of percutaneous transthoracic needle aspiration biopsy. Acta Radiol Diag 1976;17:813–28.

[6] Greene RE. Transthoracic needle aspiration biopsy. In: Athanasoulis CA, Pfister RC, Greene RE, Robertson GH, editors. Interventional radiology. Pliladelphia: Sanders; 1982. p. 587–634.
[7] Klein JS, Zarka MA. Transthoracic needle biopsy. Radiol Clin North Am 2000;38:235–66.
[8] Richardson CM, Pointon KS, Manhire AR, et al. Percutaneous lung biopsies: a survey of UK practice based on 5444 biopsies. Br J Radiol 2002;75:731–5.
[9] Belfiore G, Filippo SD, Guida C, et al. CT-guided needle biopsy of lesions. Nucle Med Biol 1994;21:713–9.
[10] Wescott JL. Air embolism complicating percutaneous needle biopsy of the lung. Chest 1973;63. pp. 108–108.
[11] Aberle DR, Gamsu G, Golden JA. Fatal systemic arterial air embolism following lung needle aspiration. Radiology 1987;165:351–3.
[12] Cianci P, Posin JP, Shimshak RR, et al. Air embolism complicating percutaneous thin needle biopsy of lung. Chest 1987;92:749–50.
[13] Tolly TL, Feldmeier JE, Czarnecki D. Air embolism complicating percutaneous lung biopsy. AJR Am J Roentgenol 1988;150:555–6.
[14] Baker BK, Awwad EE. Computed tomography of fatal cerebral air embolism following percutaneous aspiration biopsy of the lung. JCAT 1988;12:1082–3.
[15] Worth ER, Burton RJ, Landreneau RJ, Eggers GWN, et al. Left atrial air embolism during intraoperative needle biopsy of a deep pulmonary lesion. Anesthesiology 1990;73:342–5.
[16] Wong RS, Ketai L, Temes RT, Follis FM, et al. Air embolus complicating transthoracic percutaneous needle biopsy. Ann Thorac Surg 1995;59:1010–1.
[17] Khatri S. Cerebral artery gas embolism (CAGE) following fine needle aspiration biopsy of the lung. Aust NZ J Med 1997;27. pp. 27–27.
[18] Regge D, Gallo T, Galli J, et al. Systemic arterial air embolism and tension pneumothorax: two complications of transthoracic percutaneous thin-needle biopsy in the same patient. Eur Radiol 1997;7:173–5.
[19] Kodama F, Ogawa T, Hashimoto M, et al. Fatal air embolism as a complication of CT-guided needle biopsy of the lung. JCAT 1999;23:949–51.
[20] Shetty PG, Fatterpekar GM, Manohar S, et al. Fat cerebral air embolism as a complication of transbronchoscopic lung biopsy: a case report. Aust Radiol 2001;45:215–7.
[21] Arnold BW, Zwiebel WJ. Percutaneous transthoracic needle biopsy complicated by air embolism. AJR Am J Roentgenol 2002;178:1400–2.
[22] Mokhlesi B, Ansaarie I, Bazen B, et al. Coronary artery air embolism complicating a CT-guided transthoracic needle biopsy of the lung. Chest 2002;121:993–6.
[23] Laurent F, Montaudon M, Latrabe V, et al. Percutaneous biopsy in lung cancer. Eur J Radiol 2003;45:60–8.
[24] Ohi S, Ito Y, Keiya H, et al. Air embolism following computed tomography-guided lung needle marking; report of a case. Kyobu-Geka 2004;57:421–3.
[25] Kamiyoshihara M, Sakata K, Ishikawa S, et al. Cerebral arterial air embolism following CT-guided lung needle marking; report of a case. J Cardiovasc Surg 2001;42:699–700.
[26] Sakiyama S, Kondo K, Matsuoka H, et al. Fatal air embolism during computed tomography-guided pulmonary marking with a hook-type maker. J Thorac Cardiovasc Surg 2003;126:1207–9.
[27] Muller NL, Bergin CJ, Miller RR, et al. Seeding of malignant cells into the needle track after lung and pleural biopsy. J Can Assoc Radiol 1986;37:192–4.
[28] Redwood N, Beggs D, Morgan WE. Dissemination of tumor cells from fine needle biopsy. Thorax 1989;44:826–7.
[29] Berger RL, Dargan EL, Huang BL, et al. Dissemination of cancer cells by needle biopsy of the lung. J Thor Cardiovasc Surg 1972;63:430–2.
[30] Freise G, Larios R, Takeno Y, et al. Cell dissemination and implantation of neoplasms through biopsy and excision of malignant tumors. Dis Chest 1967;52:485–9.
[31] Christensen ES. Iatrogenic dissemination of tumor cells. Dissemination of tumour cells along the needle track after percutaneous, transthoracic lung biopsy. Danish Med Bull 1978;25:82–7.
[32] Ferrucci JT, Wittenberg J, Margolies MN, et al. Malignant seeding of the tract after thin-needle aspiration biopsy. Radiology 1979;130:345–6.
[33] Yoshikawa T, Yoshida J, Nishimura M, et al. Lung cancer implantation in the chest wall following percutaneous fine needle aspiration biopsy. Jpn J Clin Oncol 2000;30:450–2.
[34] Kara M, Alver G, Sak SD, Kavukcu S. Implantation metastasis caused by fine needle aspiration biopsy following curative resection of stage IB non-small cell lung cancer. Eur J Cardiothor Surg 2001;20:868–70.
[35] Ayar D, Golla B, Lee JY, Nath H. Needle-track metastasis after transthoracic needle biopsy. J Thorac Imag 1998;13:2–6.

Oncology Research, Vol. 16, pp. 85–95
Printed in the USA.

0965-0407/06 $90.00 + .00
E-ISSN 1555-3906
www.cognizantcommunication.com

Schedule-Dependent Interactions Between Pemetrexed and Cisplatin in Human Carcinoma Cell Lines In Vitro

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(Submitted July 1, 2005; revision received December 26, 2005; accepted January 10, 2006)

The combination of pemetrexed and cisplatin shows good clinical activity against mesothelioma and lung cancer. In order to study the potential cellular basis for this, and provide leads as to how to optimize the combination, we studied the schedule-dependent cytotoxic effects of pemetrexed and cisplatin against four human cancer cell lines in vitro. Tumor cells were incubated with pemetrexed and cisplatin for 24 h at various schedules. The combination effects after 5 days were analyzed by the isobologram method. Both simultaneous exposure to pemetrexed and cisplatin for 24 h and sequential exposure to cisplatin for 24 h followed by pemetrexed for 24 h produced antagonistic effects in human lung cancer A549, breast cancer MCF7, and ovarian cancer PA1 cells and additive effects in colon cancer WiDr cells. Pemetrexed for 24 h followed by cisplatin for 24 h produced synergistic effects in MCF7 cells, additive/synergistic effects in A549 and PA1 cells, and additive effects in WiDr cells. Cell cycle analysis of MCF7 and PA1 cells supported these findings. Our results suggest that the simultaneous clinical administration of pemetrexed and cisplatin may be suboptimal. The optimal schedule of pemetrexed in combination with cisplatin at the cellular level is the sequential administration of pemetrexed followed by cisplatin and this schedule is worthy of clinical investigations.

Key words: Pemetrexed; Cisplatin; Isobologram; Synergism; Antagonism

INTRODUCTION

Pemetrexed (multitargeted antifolate) is a novel antifolate that inhibits multiple points in folate metabolism including thymidylate synthase, dihydrofolate reductase, and glycinamide ribonucleotide formyl transferase (1–3). Preclinical studies of pemetrexed have demonstrated antitumor activity against a variety of human cancer cells in preclinical models (4). The optimal dose and schedule of pemetrexed was considered to be 500 mg/m^2 in a 10-min infusion once every 3 weeks (5,6). Clinical trials of pemetrexed showed a broad activity against a variety of solid tumors including malignant mesothelioma, and colorectal, pancreas, lung, head and neck, gastric, bladder, and breast cancers (6–14). Dose-limiting toxicities included neutropenia, mucositis, diarrhea, and severe nausea and vomiting (5,6). Patients with a folate-deficient state were associated with severe toxicity, and folate and cobalamin administration before pemetrexed has been introduced in clinical trials (9,13).

Combination chemotherapy has become a standard in the treatment of cancer, based upon theoretical advantages and on proven clinical efficacy. The clinical studies of pemetrexed and platinums (e.g., cisplatin, carboplatin, and oxaliplatin) in combinations have been used against malignant mesothelioma and non-small cell lung cancer, and the promising activity of this combination has been observed (15–19). The wide range of antitumor activity of pemetrexed and platinums (20), their different cytotoxic mechanisms and different toxic profiles, and the absence of cross-resistance provide a rationale for using combinations of these agents.

The cytotoxic action of cisplatin is considered to be the result of the formation of cisplatin–DNA adducts

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(20). Pemetrexed treatment may influence adduct formation by cisplatin or the repair of formed adducts, because pemetrexed inhibits both pyrimidine and purine synthesis. The disturbances of the cell cycle produced by pemetrexed and cisplatin may also influence the cytotoxic effects of each other because these agents are cell cycle specific (21,22).

These suggest that the drug schedule may play a significant role in the outcome, and therefore the design of a protocol using them in combination may requires careful consideration. Schedule-dependent interactions have been observed for the combinations of pemetrexed and gemcitabine (23), doxorubicin (24), or paclitaxel (25) in in vitro studies. Because experimental studies for the combination of pemetrexed with cisplatin are limited (26, 27), the optimal schedule of this combination is obscure.

The present study aimed at elucidating the cytotoxic effects of combinations of pemetrexed and cisplatin in various schedules on four human carcinoma cell lines. Our data suggest that the simultaneous administration of pemetrexed and cisplatin may be suboptimal for this combination and the optimal schedule of this combination at the cellular level is the sequential administration of pemetrexed followed by cisplatin.

MATERIALS AND METHODS

Cell Lines

The human lung cancer A549, the breast cancer MCF7, the ovarian cancer PA1, and the colon cancer WiDr cells were used. These cells were obtained from the American Type Culture Collection (Rockville, MD) and maintained in RPMI-1640 medium (Sigma Chemical Co., St Louis, MO) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Grand Island Biological Co.) and antibiotics. The doubling times of A549, MCF7, PA1, and WiDr cells in our experimental conditions were 20–24 h.

Drugs

Pemetrexed was kindly provided by Eli Lilly and Company (Indianapolis, IN). Cisplatin was purchased from Nihon Kayaku Co. (Tokyo). Drugs were diluted with RPMI-1640 plus 10% FBS.

Cell Growth Inhibition Using Combined Anticancer Agents

On day 0, cells growing in the exponential phase were harvested with 0.05% trypsin and 0.02% EDTA and resuspended to a final concentration of 5.0×10^3 cells/ml in fresh medium containing 10% FBS and antibiotics. The cell suspensions (100 μl) were dispensed using a multichannel pipette into the individual wells of a 96-well tissue culture plate with a lid (Falcon, Oxnard, CA). Each plate had one 8-well control column containing medium alone and one 8-well control column containing cells without drug. Eight plates were prepared for each drug combination. The cells were preincubated overnight to allow attachment.

Simultaneous Exposure to Pemetrexed and Cisplatin

After 16–20-h incubation for cell attachment, solutions of pemetrexed and cisplatin (50 μl) at different concentrations were added to the individual wells. The plates were also incubated under the same conditions for 24 h. The cells were then washed twice with culture medium containing 1% FBS, and then fresh medium containing 10% FBS (200 μl) and antibiotics was added. The cells were incubated again for 4 days.

Sequential Exposure to Pemetrexed Followed by Cisplatin or Vice Versa

After 16–20-h incubation, medium containing 10% FBS (50 μl) and solutions (50 μl) of pemetrexed (or cisplatin) at different concentrations was added to the individual wells. The plates were then incubated under the same conditions for 24 h. The cells were washed

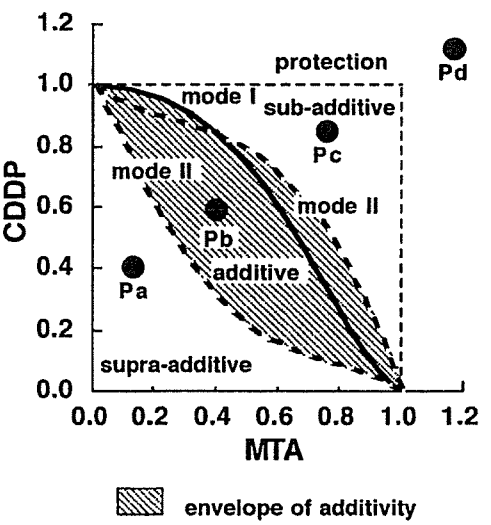

Figure 1. Schematic representation of an isobologram (29). The envelope of additivity, surrounded by mode I (solid line) and mode II (dotted lines) isobologram lines, was constructed from the dose–response curves of pemetrexed (MTA) and cisplatin (CDDP). The concentrations that produced 80% cell growth inhibition were expressed as 1.0 in the ordinate and the abscissa of all isobolograms for MCF7, PA1, and WiDr cells, while the concentrations that produced 50% cell growth inhibition were expressed as 1.0 in the ordinate and the abscissa of all isobolograms for A549 cells. The combined data points Pa, Pb, Pc, and Pd show supra-additive, additive, subadditive, and protective effects, respectively.

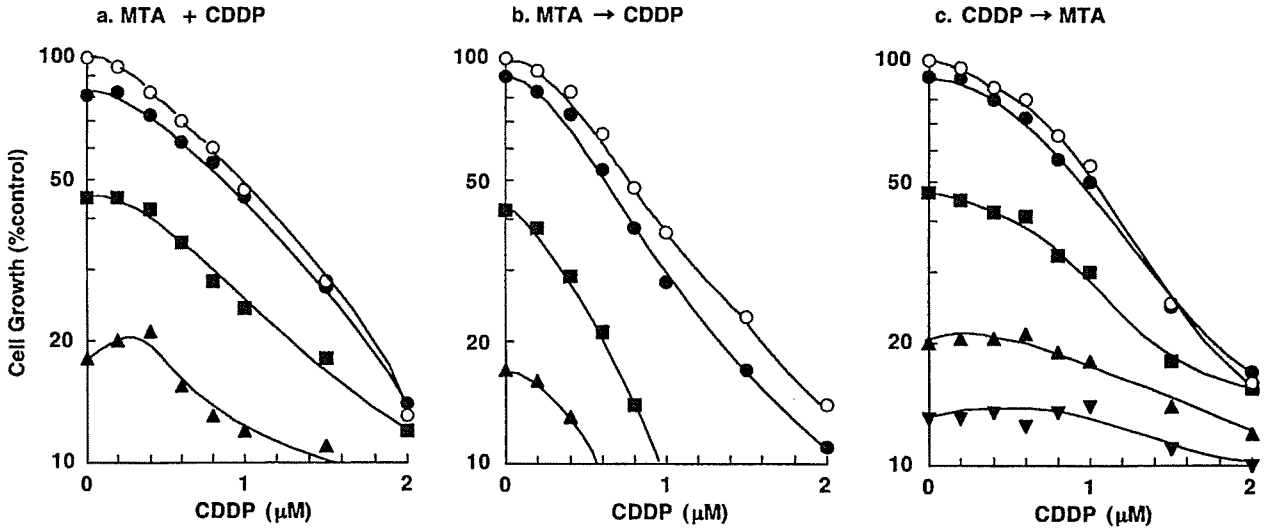

Figure 2. Schedule dependence of the interaction between pemetrexed and cisplatin in PA1 cells. Cells were exposed to these two drugs simultaneously for 24 h (a), pemetrexed first for 24 h followed by cisplatin for 24 h (b), or the reverse sequence (c). The cell number after 5 days was measured using the MTT assay and was plotted as a percentage of the control (cells not exposed to drugs). The concentrations of cisplatin are shown on the abscissa. The concentrations of pemetrexed were 0 (open circles), 20 (filled circles), 50 (filled squares), 100 (filled upward triangles), and 200 (filled downward triangles) nM, respectively. Data are mean values for three independent experiments; SE was <20%.

twice with culture medium containing 1% FBS; fresh medium containing 10% FBS (150 μl) and antibiotics was added, followed by the addition of solutions (50 μl) of cisplatin (or pemetrexed) at different concentrations. The plates were incubated again under the same conditions for 24 h. The cells were then washed twice with culture medium, and fresh medium containing 10% FBS (200 μl) and antibiotics was added. The cells were then incubated again for 3 days.

MTT Assay

The cytotoxicity of pemetrexed alone, cisplatin alone, and their combinations was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay as described previously (28). For all four cell lines examined, we were able to establish a linear relationship between the MTT assay value and the cell number within the range shown.

Isobologram

The dose–response interactions between pemetrexed and cisplatin for the MCF7, PA1, and WiDr cells were evaluated at the IC_{80} level by the isobologram method of Steel and Peckham (Fig. 1) (29). The IC_{80} was defined as the concentration of drug that produced 80% cell growth inhibition (i.e., an 80% reduction in absorbance). Although the drug interaction at IC_{90} or more would be more important than both IC_{80} and IC_{50} for cancer chemotherapy, it is difficult to get reliable data at IC_{90} or more using MTT assay. A549 was resistant to pemetrexed and the interactions between them were evaluated at the IC_{50} level.

We used the isobologram method of Steel and Peckham because this method can cope with any agents with unclear cytotoxic mechanisms and a variety of dose–response curves of anticancer agents. The concept and analysis of the isobologram has been described in detail previously (30,31). The isobologram of Steel and Peckham is very strict for synergism and antagonism.

If the two agents act additively by independent mechanisms, the combined data points would lie near the mode I line (hetero-addition). If the agents act additively by similar mechanisms, the combined data points would lie near the mode II lines (iso-addition). When the data points of the drug combination fell within the area surrounded by mode I and /or mode II lines (i.e., within the envelope of additivity), the combination was described as additive.

A combination that gives data points to the left of the envelope of additivity (i.e., the combined effect is caused by lower doses of the two agents than is predicted) can confidently be described as supra-additive (synergism). A combination that gives data points to the right of the envelope of additivity, but within the square or on the line of the square, can be described as subadditive (i.e., the combination is superior or equal to a single agent but is less than additive). A combination that gives

data points outside the square can be described as protective (i.e., the combination is inferior in cytotoxic action to a single agent). A combination with both subadditive and/or protective interactions can confidently be described as antagonistic.

Data Analysis

The findings were analyzed as described previously (32). When the observed data points from combinations fell mainly in the area of supra-additivity or in the areas of subadditivity and protection, the mean value of the observed data was smaller than that of the predicted minimum data or larger than that of the predicted maximum data, the combinations were considered to have a synergistic or an antagonistic effect, respectively. To determine whether the condition of synergism (or antagonism) truly existed, a Wilcoxon signed-rank test was performed to compare the observed data with the predicted minimum (or maximum) data for an additive effect. Probability values of $p < 0.05$ were considered significant. Because the isobologram of Steel and Peckham is very strict for synergism and antagonism, combinations with $p \geq 0.05$ were defined as having an additive/synergistic (or additive/antagonistic) effect. All statistical analyses were performed using the Stat View 4.01 software program (Abacus Concepts, Berkeley, CA).

Flow Cytometric Analysis

PA1 cells were treated with 0.2 μM pemetrexed alone or 0.5 μM cisplatin alone or their combination simultaneously for 24 h. MCF7 cells were treated with 0.5 μM pemetrexed alone or 5 μM cisplatin alone or their combination simultaneously for 24 h. The cells were also treated with pemetrexed for 24 h followed by cisplatin for 24 h or the reverse sequence. The cells were harvested at 48 h and the cell cycle profiles were analyzed by staining the intracellular DNA with propidium iodide in preparation for flow cytometry with the FACScan CellFIT system (Becton-Dickinson, San Jose, CA). A DNA histogram was obtained by analyzing 25,000 cells with the ModFIT program (Becton-Dickinson) (33).

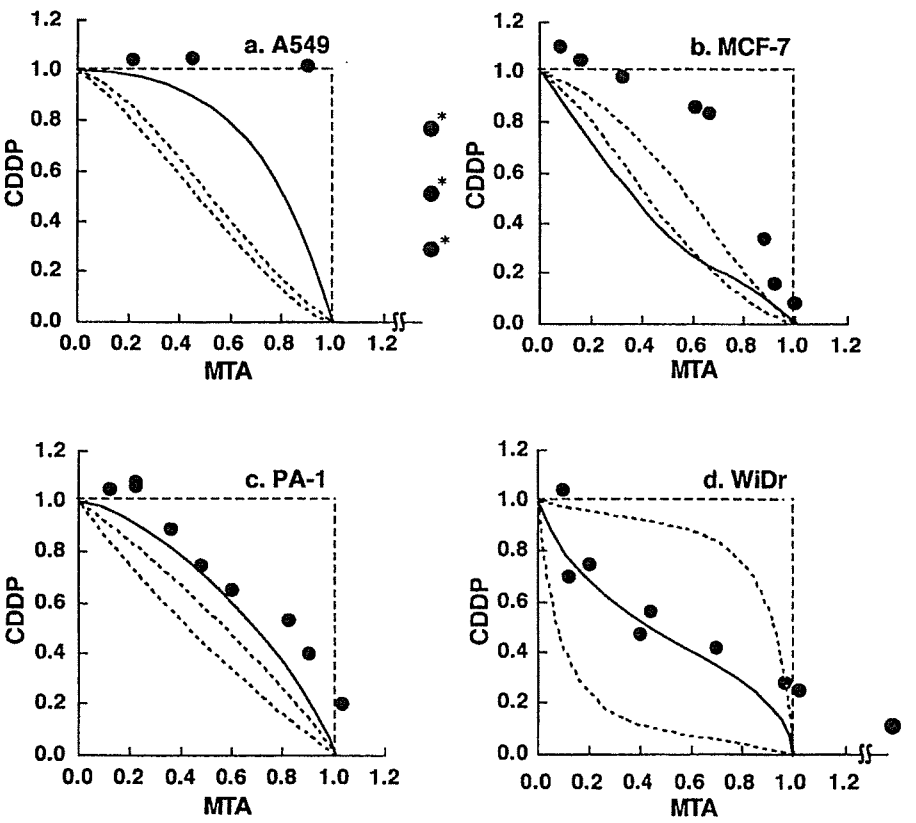

Figure 3. Isobolograms of simultaneous exposure to pemetrexed and cisplatin for 24 h in A549 (a), MCF7 (b), PA1 (c), and WiDr (d) cells. For the A549, MCF7, and PA1 cells, the combined data points fell in the areas of subadditivity and protection. For the WiDr cells, the combined data points fell mainly within the envelope of additivity. Data are mean values for at least three independent experiments; SE was <25% (*except the data).

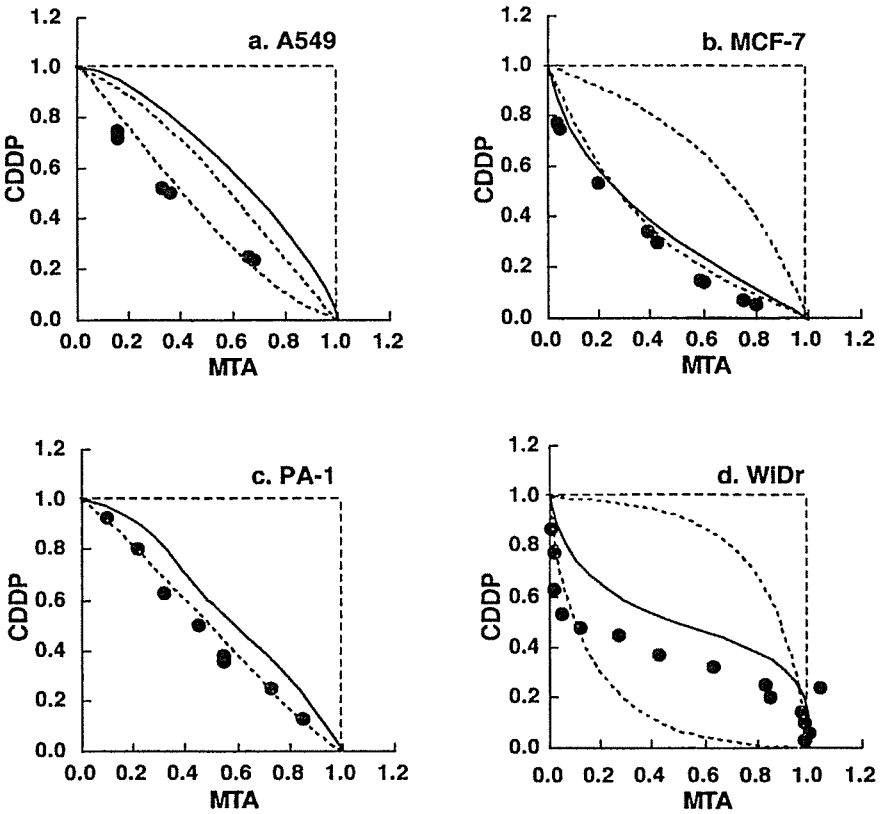

Figure 4. Isobolograms of sequential exposure to pemetrexed (24 h) followed by cisplatin (24 h) in A549 (a), MCF7 (b), PA1 (c), and WiDr (d) cells. For the A549, MCF7, and PA1 cells, all or most of the data points of the combinations fell within the envelope of additivity and in the area of supra-additivity. For the WiDr cells, most of the data points fell within the envelope of additivity. Data are mean values for at least three independent experiments; SE was <20%.

RESULTS

The IC_{80} values of 24-h exposure to pemetrexed for A549, MCF7, PA1, and WiDr cells were >5, 2.5 ± 0.4, 0.10 ± 0.03, and 0.55 ± 0.2 μM, respectively. Because A549 cells were resistant to pemetrexed and the IC_{80} level was not obtained, the interactions between pemetrexed and cisplatin were evaluated at the IC_{50} level. The IC_{50} value of 24-h exposure to pemetrexed for A549 cells was 2.7 ± 0.3 μM.

Figure 2 shows the dose–response curves obtained from simultaneous exposure and sequential exposure to pemetrexed and cisplatin for the PA1 cells. The dose–response curves were plotted on a semilog scale as a percentage of the control, the cell number of which was obtained from the samples not exposed to the drugs administered simultaneously. Dose–response curves in which the pemetrexed concentrations are shown on the abscissa could be made based on the same data (figure not shown). Based upon the dose–response curves of pemetrexed alone and cisplatin alone, three isoeffect curves (mode I and mode II lines) were constructed. Isobolograms at the IC_{80} or IC_{50} levels were generated based upon these dose–response curves for the combinations.

Simultaneous Exposure to Pemetrexed and Cisplatin

Figure 3 shows isobolograms of the A549, MCF7, PA1, and WiDr cells after simultaneous exposure to pemetrexed and cisplatin for 24 h. For the A549, MCF7, and PA1 cells, the combined data points fell in the areas of subadditivity and protection, respectively. The mean values of the observed data (>1.15, 0.95, and 0.69) were larger than those of the predicted maximum values (0.75, 0.72, and 0.56). The observed data and the predicted maximum data were compared by the Wilcoxon signed-rank test. The differences were significant ($p < 0.05$, $p < 0.02$, and $p < 0.01$), indicating antagonistic effects (Table 1). For the WiDr cells, the combined data points fell mainly within the envelope of additivity. The mean values of the observed data (0.66) were larger than those of the predicted minimum values (0.27), and smaller than those of the predicted maximum values (0.73), indicating additive effects.

Sequential Exposure to Pemetrexed Followed by Cisplatin

Figure 4 shows isobolograms of the A549, MCF7, PA1, and WiDr cells exposed first to pemetrexed for 24 h and then cisplatin for 24 h. For the MCF7 cells, combined data points fell in the area of supra-additivity. The mean values of the observed data (0.40) were smaller than those of the predicted minimum values (0.44) (Table 1). The difference between them was significant ($p < 0.01$), indicating synergistic effects. For the A549 and PA1 cells, combined data points fell in the area of supra-additivity and within the envelope of additivity. The mean values of the observed data were smaller than those of the predicted minimum values (Table 1), but the differences were not significant ($p > 0.05$ and $p > 0.05$), indicating additive/synergistic effects. For the WiDr cells, the combined data points fell within the envelope of additivity and in the areas of supra-additivity and protection. The mean value of the observed data was smaller than the predicted maximum values and larger than that of the predicted minimum values (Table 1), indicating additive effects.

Sequential Exposure to Cisplatin Followed by Pemetrexed

Figure 5 shows isobolograms of the four cell lines exposed first to cisplatin for 24 h and then pemetrexed for 24 h. For the A549, MCF7, and PA1 cells, all or most of the combined data points fell in the areas of subadditivity and protection. The mean values of the observed data were larger than those of the predicted maximum values (Table 1). The differences were significant ($p < 0.05$, $p < 0.02$, and $p < 0.02$, respectively), indicating antagonistic effects. For the WiDr cells, most of the combined data points fell within the envelope of additivity, indicating an additive effect of this schedule.

Flow Cytometric Analysis

Finally, we evaluated the cytotoxic effects of pemetrexed and cisplatin on cancer cells using flow cytome-

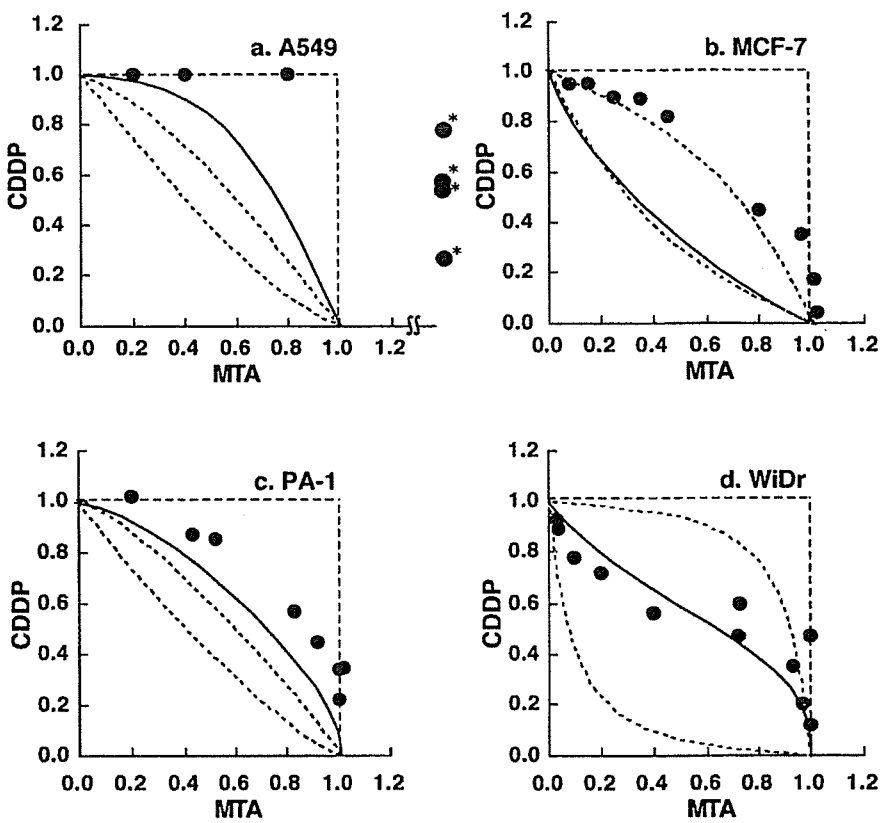

Figure 5. Isobolograms of sequential exposure to cisplatin (24 h) followed by pemetrexed (24 h) in A549 (a), MCF7 (b), PA1 (c), and WiDr (d) cells. For the A549, MCF7, and PA1 cells, all or most of the data points of the combinations fell in the areas of subadditivity and protection. For the WiDr cells, most of the data points of the combinations fell within the envelope of additivity and in the area of subadditivity. Data are mean values for at least three independent experiments; SE was <20%(*except the data).

Table 1. Mean Values of Observed, Predicted Minimum, and Predicted Maximum Data of Pemetrexed (MTA) in Combination With Cisplatin (CDDP) at IC_{80} for MCF7, PA1, and WiDr Cells and at IC_{50} for A549 Cells

Schedule	Cell Line	n	Observed Data	Predictaed Data for an Additive Effect		Effect
				Minimum	Maximum	
MTA + CDDP	A549	6	1.15	0.44	0.75	antagonism ($p < 0.05$)
	MCF7	8	0.95	0.57	0.72	antagonism ($p < 0.02$)
	PA1	9	0.69	0.40	0.56	antagonism ($p < 0.01$)
	WiDr	9	0.66	0.27	0.73	additive
MTA → CDDP	A549+	6	0.45	0.47	0.72	additive/synergism ($p > 0.05$)
	MCF7	9	0.40	0.44	0.78	synergism ($p < 0.01$)
	PA1	8	0.52	0.55	0.64	additive/synergism($p > 0.05$)
	WiDr	15	0.64	0.46	0.84	additive
CDDP → MTA	A549	7	1.14	0.41	0.74	antagonism ($p < 0.05$)
	MCF7	9	0.82	0.52	0.73	antagonism ($p < 0.02$)
	PA1	8	0.75	0.41	0.63	antagonism ($p < 0.02$)
	WiDr	11	0.71	0.21	0.82	additive

try. Cell cycle analysis revealed that pemetrexed and cisplatin arrested PA1 cells in late G_1 to early S phase and G_2/M phase, respectively (Fig. 6A, Table 2). When PA1 cells were exposed to both drugs simultaneously, the cell cycle profile was almost identical to that of a single treatment with pemetrexed, suggesting that the cell cycle effect of pemetrexed is dominant over that of cisplatin. As a result, the apoptosis-inducing effect of cisplatin, which was estimated by an increase in the size of sub-G_1 fraction, was almost completely cancelled in the presence of pemetrexed (Fig. 6A, MTA + CDDP). When PA1 cells were treated with cisplatin first and followed by pemetrexed, the cell cycle pattern closely resembled that of cells treated with cisplatin alone except for a modest increase in G_1 and S phases (Fig. 6A, Table 2, CDDP to MTA). The induction of apoptotsis was less prominent in the CDDP to MTA treatment than in the CDDP treatment (Table 2). In contrast, both apoptosis and G_2/M arrest were enhanced when PA1 cells were treated with pemetrexed first and followed by cisplatin compared with the treatment with either pemetrexed or cisplatin alone (Fig. 6A, Table 2, MTA to CDDP).

We carried out the same analysis with another cancer cell line MCF7 and obtained highly reproducible results. Upon simultaneous addition, the cell cycle effect of cisplatin was almost completely abrogated and the percentage of apoptotic cells was less than that of a single treatment with pemetrexed (Fig. 6B, MTA + CDDP). Similarly, apoptosis was suppressed when MCF7 cells were treated with cisplatin first and followed by pemetrexed compared with the treatment with either pemetrexed or cisplatin alone (Fig. 6B, Table 2, CDDP to MTA). In contrast, the apoptosis-inducing effect of pemetrexed was enhanced by the sequential exposure to cisplatin after pemetrexed (Fig. 6B, Table 2, MTA to CDDP). Overall, these data are fully consistent with the results of isobologram analysis, and provide the molecular basis of the interaction between the two drugs.

DISCUSSION

We found that the cytotoxic interaction between pemetrexed and cisplatin was schedule dependent. Simultaneous exposure to pemetrexed and cisplatin and sequential exposure to cisplatin followed by pemetrexed showed antagonistic effects in A549, MCF7, and PA1 cells, while sequential exposure to pemetrexed followed by cisplatin had a tendency to produce synergistic effects. In the latter schedule, observed data points in A549, MCF7, and PA1 cells were smaller than predicted minimum values for an additive effect (Table 1). WiDr cells showed additive effects in all schedules. The cause of difference in combined effects among cell lines is unknown. The difference may reflect the folate metabolism and the variety of target numbers (enzymes) in the cells. In addition, the isobologram of Steel and Peckham is stricter for synergism and antagonism than other methods. This may also influence the results.

In general, it is difficult to clarify the mechanisms underlying the drug combination. In this study, however, cell cycle analysis provided a clue to understand the molecular basis of schedule-dependent synergism and antagonism of the combination of pemetrexed and cisplatin. The exposure of PA1 and MCF7 cells to pemetrexed for 24 h led to a synchronization of most cells in late G_1 to

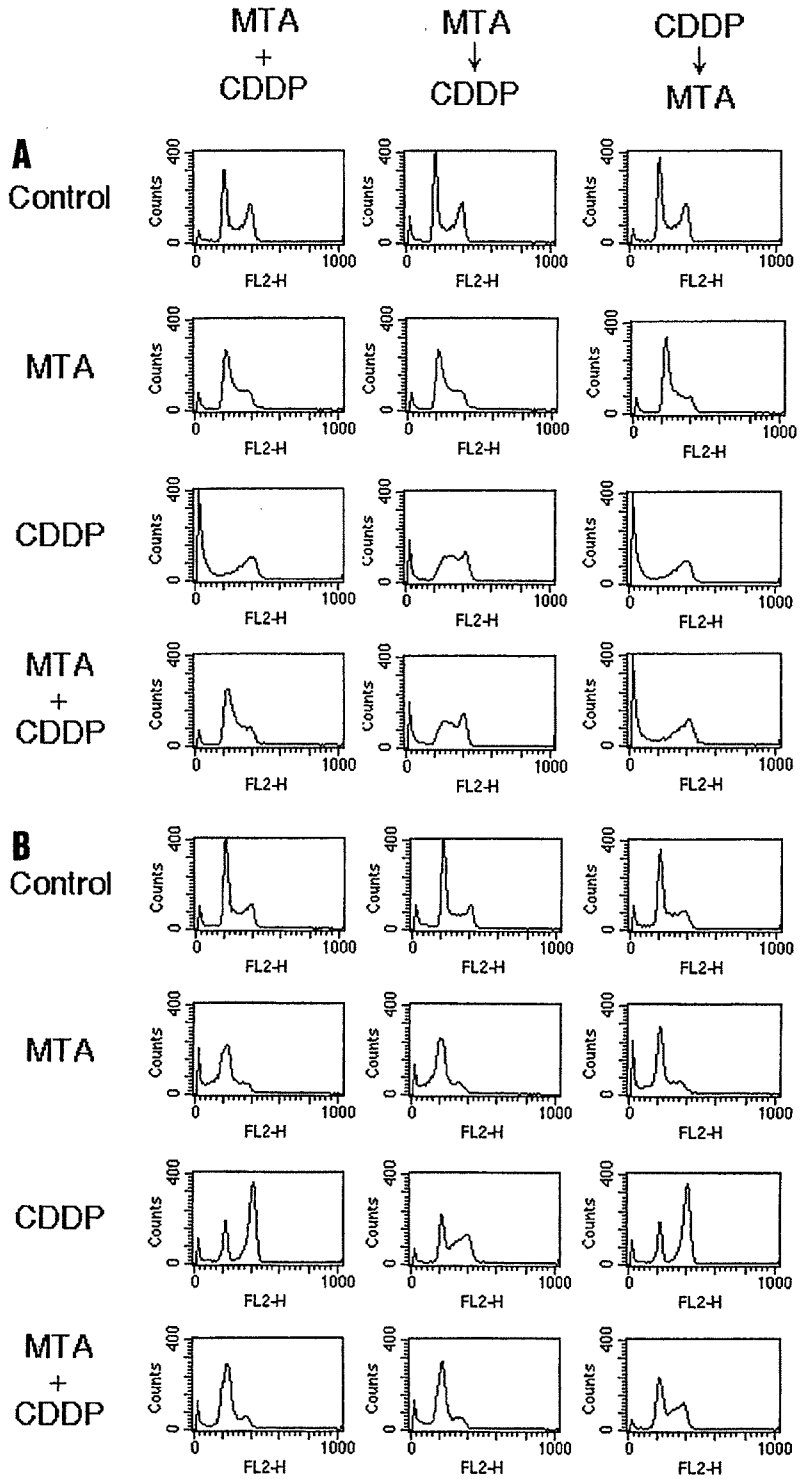

Figure 6. Flow cytometric analysis of cell cycle perturbation. PA1 cells, treated with 0.2 μM pemetrexed (MTA), 0.5 μM cisplatin (CDDP), both drugs simultaneously for 24 h, pemetrexed for 24 h followed by cisplatin for 24 h, or the reverse sequence were harvested at 48 h (A), and MCF7 cells, treated with 0.5 μM pemetrexed (MTA), 5 μM cisplatin (CDDP), both drugs simultaneously for 24 h, pemetrexed for 24 h followed by cisplatin for 24 h, or the reverse sequence were harvested at 48 h (B) and stained for DNA with propidium iodide and analyzed by flow cytometry as described in Materials and Methods.

Table 2. Cell Cycle Perturbations Induced by Pemetrexed (MTA), Cisplatin (CDDP), and Their Combinations for PA1 and MCF7 Cells at 48 h

	MTA + CDDP (24 h)				MTA (24 h) → CDDP (24 h)				CDDP (24 h)→ MTA (24 h)			
Cell Cycle (%)	Control	MTA	CDDP	MTA + CDDP	Control	MTA	CDDP	MTA + CDDP	Control	MTA	CDDP	MTA + CDDP
PA1 cells												
Sub-G_1	3.6	2.4	42.9	2.1	4.3	3.1	8.9	15.3	2.9	2.2	45.1	41.8
G_1	56.2	64.1	7.3	67.1	58.1	65.3	5.8	4.4	57.3	60.1	6.9	10.6
S	15.6	26.7	17.2	19.1	10.4	25.9	48.4	38.7	11.0	30.4	15.8	20.1
G_2/M	24.6	6.8	19.1	11.7	27.2	5.7	36.9	41.6	28.8	7.3	32.2	27.5
MCF-7 cells												
Sub-G_1	4.2	17.5	3.9	5.8	5.3	11.1	2.9	16.8	5.1	10.3	3.6	2.5
G_1	57.6	53.4	28.8	63.7	55.8	61.3	22.3	60.6	58.8	57.2	27.9	25.8
S	16.8	26.9	4.7	21.4	19.1	22.1	21.2	13.8	16.4	28.6	5.0	20.4
G_2/M	21.4	2.2	62.6	9.1	25.1	5.5	53.6	8.8	19.7	3.9	63.5	51.3

early S phase, in which cells are sensitive to cisplatin (20). This may explain the synergistic effects of sequential exposure to pemetrexed followed by cisplatin. On the contrary, one agent may reduce the cytotoxicity of the other agent by preventing cells from entering the specific phase in which the cells are most cytotoxic to the other agent. It has been shown that cisplatin elicits cytotoxic effects by blocking cells in G_2/M phase (20), while pemetrexed does by blocking cells in S phase (21). Indeed, simultaneous exposure to pemetrexed and cisplatin produced antagonistic effects, which were caused by the cancellation of cisplatin-induced G_2/M arrest by coexisting pemetrexed in PA1 and MCF7 cells. This was also the case with sequential exposure with cisplatin first followed by pemetrexed.

Our findings suggest that the sequential administration of pemetrexed followed by cisplatin may be the optimal schedule for these combinations. For example, administrations of pemetrexed on day 1 and cisplatin on day 2 would be worthy of clinical investigations. The simultaneous administration of pemetrexed and cisplatin and the sequential administration of cisplatin followed by pemetrexed may be inadequate. However, it must be noted that there are a number of difficulties in the translation of results from in vitro models to clinical therapy. The drug metabolism and pharmacokinetics under in vivo and in vitro conditions are different. Clinical outcome includes both the antitumor effects and normal tissue toxicity that results from a variable drug exposure, whereas in vitro models represent only antitumor effects at a constant drug exposure.

Teicher et al. studied the combination of pemetrexed with cisplatin in vivo against EMT-6 murine mammary carcinoma by a tumor cell survival assay (26). They observed that pemetrexed administered four times over 48 h with cisplatin administered with the third dose of pemetrexed produced an additive or more than additive tumor response. Teicher et al. further studied the combination of pemetrexed with cisplatin in human tumor xenografts (27). Administration of pemetrexed (days 7–11, days 14–18) along with cisplatin (day 7) produced greater-than-additive effects for human lung cancer H460 and Calu-6 tumor growth delay. Because experimental systems, schedules of drug administrations, and evaluating methods for synergism are different, it is difficult to compare their findings and ours.

A clinical and pharmacokinetic phase I study of pemetrexed in combination with cisplatin has been reported by Thordtmann et al. (15). They observed that this combination was clinically active and simultaneous administration of both agents on day on 1 (pemetrexed intravenously over 10 min and cisplatin over 2 h) every 21 days was less toxic than a sequential administration of pemetrexed on day 1 and cisplatin on day 2. They recommended the simultaneous administration of pemetrexed at 500 mg/m^2 plus cisplatin at 75 mg/m^2 on day 1 every 21 days for this combination. Phase II and III studies of the same schedules have been started for this combination and encouraging results have been obtained so far (16–18).

Our in vitro findings are not contradictory to clinical findings. In our study, simultaneous exposure to pemetrexed and cisplatin produced additive effects in WiDr cells and antagonistic effects in A549, MCF7, andPA1 cells. Most data points fell in the area of sudadditivity in MCF7 and PA1 cells, suggesting that the combination is superior to each drug alone but "sub-optimal." The simultaneous administration of pemetrexed and cisplatin was less toxic than the sequential administration, probably due to antagonistic interaction in the simultaneous exposure. Our isobologram shows that the doses of both agents in the pemetrexed–cisplatin sequence required

for IC_{80} or IC_{50} levels were much less (40–90%) than of those in simultaneous exposure (Fig. 3). Pemetrexed at 500 mg/m^2 and cisplatin at 75 mg/m^2, the optimal dose for the simultaneous administration, would be overdosed for the sequential administration of pemetrexed followed by cisplatin, which produced synergistic effects.

In conclusion, the present findings show that the interaction of pemetrexed and cisplatin is definitely schedule dependent. Sequential exposure to pemetrexed followed by cisplatin produced synergistic effects, whereas simultaneous exposure to the two agents and sequential exposure to cisplatin followed by pemetrexed produced antagonistic effects. These findings suggest that the optimal schedule of pemetrexed in combination with cisplatin at the cellular level is the sequential administration of pemetrexed followed by cisplatin. Although the simultaneous administration of pemetrexed and cisplatin on day 1 is more convenient and less toxic for patients than the sequential administration of pemetrexed on day 1 and cisplatin on day 2, the former schedule may be suboptimal and may not improve the clinical efficacy to "originally expected" level for this combination. It would be important to conduct dose-finding clinical trials in sequential administration of pemetrexed and cisplatin.

ACKNOWLEDGMENTS: This work was supported in part by a Grant-in-Aid for Cancer Research (11-8) from the Ministry of Health and Welfare and by a Grant-in-Aid for Research on the Second-Term Comprehensive 10-Year Strategy for Cancer Control from the Ministry of Health and Welfare of Japan.

REFERENCES

1. Taylor, E. C.; Kuhnt, D.; Shih, C.; Rinzel, S. M.; Grindey, G. B.; Barredo, J.; Jannatipour, M.; Moran, R. G. A dideazatetrahydrofolate analogue lacking a chiral center at C-6 N-[4-[2-(2-amino-34-dihydro-4-oxo-7H-pyrrolo[23-d]pyrimidin-5- yl)ethyl]benzoyl]-L-glutamic acid is an inhibitor of thymidylate synthase. J. Med. Chem. 35:4450–4454; 1992.
2. Habeck, L. L.; Mendelsohn, L. G.; Shih, C.; Taylor, E. C.; Colman, P. D.; Gossett, L. S.; Leitner, T. A.; Schultz, R. M.; Andis, S. L.; Moran, R. G. Substrate specificity of mammalian folypolyglutamate synthetase for 510-dideazatetrahydrofolate analogs. Mol. Pharmacol. 48: 326–333; 1995.
3. Shih, C.; Habeck, L. L.; Mendelsohn, L. G.; Chen, V. J.; Schultz, R. M. Multiple folate enzyme inhibition: Mechanism of a novel pyrrolopyrimidine-based antifolate LY231514 (MTA). Adv. Enzyme Regul. 38:135–152; 1998.
4. Shih, C.; Thornton, D. E. Preclinical pharmacology studies and the clinical development of a novelmultitargeted antifolate MTA (LY231514) In: Jackman, A. L., ed. Anticancer drug development guide: Antifolate drugs in cancer therapy. Totowa, NJ: Humana Press; 1998:183–201.
5. McDonald, A. C.; Vasey, P. A.; Adams, L.; Walling, J.; Woodworth, J. R.; Abrahams, T.; McCarthy, S.; Bailey, N. P.; Siddiqui, N.; Lind, M. J.; Calvert, A. H.; Twelves, C. J.; Cassidy, J.; Kaye, S. B. A phase I and pharmacokinetic study of LY231514 the multitargeted antifolate. Clin. Cancer Res. 4:605–610; 1998.
6. Rinaldi, D. A. Overview of phase I trials of multitargeted antifolate (MTA LY231514). Semin. Oncol. 26(Suppl. 6): 82–88; 1999.
7. Rusthoven, J. J.; Eisenhauer, E.; Butts, C.; Gregg, R.; Dancey, J.; Fisher, B.; Iglesias, J. Multitargeted antifolate LY231514 as first-line chemotherapy for patients with advanced non-small-cell lung cancer: A phase II study. National Cancer Institute of Canada Clinical Trials Group. J. Clin. Oncol. 17:1194–1199; 1999.
8. John, W.; Picus, J.; Blanke, C. D.; Clark, J. W.; Schulman, L. N.; Rowinsky, E. K.; Thornton, D. E.; Loehrer, P. J. Activity of multitargeted antifolate (pemetrexed disodium LY231514) in patients with advanced colorectal carcinoma: Results from a phase II study. Cancer 88:1807–1813; 2000.
9. Hanauske, A. R.; Chen, V.; Paoletti, P.; Niyikiza, C. Pemetrexed disodium: A novel antifolate clinically active against multiple solid tumors. Oncologist 6:363–373; 2001.
10. Pivot, X.; Raymond, E.; Laguerre, B.; Degardin, M.; Cals, L.; Armand, J. P.; Lefebvre, J. L.; Gedouin, D.; Ripoche, V.; Kayitalire, L.; Niyikiza, C.; Johnson, R.; Latz, J.; Schneider, M. Pemetrexed disodium in recurrent locally advanced or metastatic squamous cell carcinoma of the head and neck. Br. J. Cancer 85:649–655; 2001.
11. Shepherd, F. A. Pemetrexed in the treatment of non-small cell lung cancer. Semin. Oncol. 29(Suppl. 18):43–48; 2002.
12. Calvert, H. Pemetrexed (Alimta): A promising new agent for the treatment of breast cancer. Semin. Oncol. 30 (Suppl. 3):2–5; 2003.
13. Scagliotti, G. V.; Shin, D. M.; Kindler, H. L.; Scagliotti, G. V.; Shin, D. M.; Kindler, H. L.; Vasconcelles, M. J.; Keppler, U.; Manegold, C.; Burris, H.; Gatzemeier, U.; Blatter, J.; Symanowski, J. T.; Rusthoven, J. J. Phase II study of pemetrexed with and without folic acid and vitamin B12 as front-line therapy in malignant therapy in malignant pleural mesothelioma. J. Clin. Oncol. 21:1556–1561; 2003.
14. Hanna, N.; Shepherd, F. A.; Fossella, F. V.; Pereira, J. R.; De Marinis, F.; von Pawel, J.; Gatzemeier, U.; Tsao, T. C.; Pless, M.; Muller, T.; Lim, H. L.; Desch, C.; Szondy, K.; Gervais, R.; Shaharyar; Manegold, C.; Paul, S.; Paoletti, P.; Einhorn, L.; Bunn, Jr., P. A. Randomized phase III trial of pemetrexed versus docetaxel in patients with non-small-cell lung cancer previously treated with chemotherapy. J. Clin. Oncol. 22:1589–1597; 2004.
15. Thodtmann, R.; Depenbrock, H.; Dumez, H.; Blatter, J.; Johnson, R. D.; van Oosterom, A.; Hanauske, A. R. Clinical and pharmacokinetic phase I study of multitargeted antifolate (LY231514) in combination with cisplatin. J. Clin. Oncol. 17:3009–3016; 1999.
16. Manegold, C.; Gatzemeier, U.; von Pawel, J.; Pirker, R.; Malayeri, R.; Blatter, J.; Krejcy, K. Front-line treatment of advanced non-small-cell lung cancer with MTA (LY231514 pemetrexed disodium ALIMTA) and cisplatin: A multicenter phase II trial. Ann. Oncol. 11:435–440; 2000.
17. Shepherd, F. A.; Dancey, J.; Arnold, A.; Neville, A.; Rusthoven, J.; Johnson, R. D.; Fisher, B.; Eisenhauer, E. Phase II study of pemetrexed disodium a multitargeted antifolate and cisplatin as first-line therapy in patients with advanced non small cell lung carcinoma: A study

of the National Cancer Institute of Canada Clinical Trials Group. Cancer 92:595–600; 2001.
18. Vogelzang, N. J.; Rusthoven, J. J.; Symanowski, J.; Denham, C.; Kaukel, E.; Ruffie, P.; Gatzemeier, U.; Boyer, M.; Emri, S.; Manegold, C.; Niyikiza, C.; Paoletti, P. Phase III study of pemetrexed in combination with cisplatin versus cisplatin alone in patients with malignant pleural mesothelioma. J. Clin. Oncol. 21:2636–2644; 2003.
19. Scagliotti, G. V.; Kortsik, C.; Dark, G. G.; Price, A.; Manegold, C.; Rosell, R.; O'Brien, M.; Peterson, P. M.; Castellano, D.; Selvaggi, G.; Novello, S.; Blatter, J.; Kayitalire, L.; Crino, L.; Paz-Ares, L.; Go, R. S. Pemetrexed combined with oxaliplatin or carboplatin as first-line treatment in advanced non-small cell lung cancer: A multicenter, randomized, phase II trial. Clin. Cancer Res. 11(2 Pt. 1):690–696; 2005.
20. Adjei, A. A. Review of the comparative pharmacology and clinical activity of cisplatin and carboplatin. J. Clin. Oncol. 17:409–422; 1999.
21. Jackel, M.; Kopf-Maier, P. Influence of cisplatin on cell-cycle progression in xenografted human head and neck carcinomas. Cancer Chemother. Pharmacol. 27:464–471; 1991.
22. Tonkinson, J. L.; Marder, P.; Andis, S. L.; Schultz, R. M.; Gossett, L. S.; Shih, C.; Mendelsohn, L. G. Cell cycle effects of antifolate antimetabolites: Implications for cytotoxicity and cytostasis. Cancer Chemother. Pharmacol. 39: 521–531; 1997.
23. Tonkinson, J. L.; Worzalla, J. F.; Teng, C. H.; Mendelsohn, L. G. Cell cycle modulation by a multitargeted antifolate, LY231514, increases the cytotoxicity and antitumor activity of gemcitabine in HT29 colon carcinoma. Cancer Res. 59:3671–3676; 1999.
24. Schultz, R. M.; Dempsey, J. A. Sequence dependence of Alimta (LY231514, MTA) combined with doxorubicin in ZR-75-1 human breast carcinoma cells. Anticancer Res. 21:3209–3214; 2001.
25. Kano, Y.; Akutsu, M.; Tsunoda, S.; Izumi, T.; Mori, K.; Fujii, H.; Yazawa, Y.; Mano, H.; Furukawa, Y. Schedule-dependent synergism and antagonism between pemetrexed and paclitaxel in human carcinoma cell lines in vitro. Cancer Chemother. Pharmacol. 54:505–513; 2004.
26. Teicher, B. A.; Alvarez, E.; Liu, P.; Lu, K.; Menon, K.; Dempsey, J.; Schultz, R. M. MTA (LY231514) in combination treatment regimens using human tumor xenografts and the EMT-6 murine mammary carcinoma. Semin. Oncol. 28:55–62; 1999.
27. Teicher, B. A.; Chen, V.; Shih, C.; Menon, K.; Forler, P. A.; Phares, V. G.; Amsrud, T. Treatment regimens including themultitargeted antifolate LY231514 in human tumor xenografts. Clin. Cancer Res. 6:1016–1023; 2000.
28. Kano, Y.; Sakamoto, S.; Kasahara, T.; Akutsu, M.; Inoue, Y.; Miura, Y. In vitro effects of amsacrine in combination with other anticancer agents. Leukemia Res. 15:1059–1064; 1991.
29. Steel, G. G.; Peckham, M. J. Exploitable mechanisms in combined radiotherapy-chemotherapy: the concept of additivity. Int. J. Radiat. Oncol. Biol. Phys. 5:85–91; 1979.
30. Kano, Y.; Ohnuma, T.; Okano, T.; Holland, J. F. Effects of vincristine in combination with methotrexate and other antitumor agents in human acute lymphoblastic leukemia cells in culture. Cancer Res. 48:351–356; 1988.
31. Kano, Y.; Akutsu, M.; Tsunoda, S.; Mano, H.; Sato, Y.; Honma, Y.; Furukawa, Y. In vitro cytotoxic effects of a tyrosine kinase inhibitor STI571 in combination with commonly used antileukemic agents. Blood 97:1999–2007; 2001.
32. Kano, Y.; Akutsu, M.; Tsunoda, S.; Suzuki, K.; Adachi, K. In vitro schedule-dependent interaction between paclitaxel and SN-38 (the active metabolite of irinotecan) in human carcinoma cell lines. Cancer Chemother. Pharmacol. 42:91–98; 1998.
33. Furukawa, Y.; Iwase, S.; Kikuchi, J.; Nakamura, M.; Terui, Y.; Yamada, H.; Kano, Y.; Matsuda, M. Phosphorylation of bcl-2 protein by cdc2 Kinase during G2/M phases and its role in cell cycle regulation. J. Biol. Chem. 275:21661–21667; 2000.

Lung Cancer (2006) 53, 197–203

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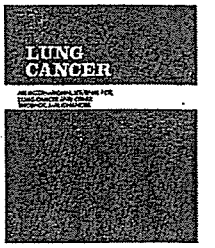

A combination chemotherapy of carboplatin and irinotecan with granulocyte colony-stimulating factor (G-CSF) support in elderly patients with small cell lung cancer☆

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Received 23 January 2006; received in revised form 23 April 2006; accepted 9 May 2006

KEYWORDS
Small cell lung cancer;
Elderly;
Chemotherapy;
Carboplatin;
Irinotecan

Summary

Background: We have previously reported that carboplatin plus etoposide is an effective and relatively non-toxic regimen in elderly patients with small cell lung cancer (SCLC). Recently, the Japan Clinical Oncology Group reported that irinotecan plus cisplatin was more effective than etoposide plus cisplatin in the treatment of non-elderly patients with extensive disease (ED)-SCLC. Therefore, we conducted a prospective feasibility study designed specifically to evaluate the efficacy of carboplatin (day 1) and irinotecan (days 1, 8, 15) with granulocyte colony-stimulating factor (G-CSF) support in elderly SCLC patients.

Methods: Three carboplatin AUC and irinotecan dose levels were used: 4 mg/ml × min and 50 mg/m^2, respectively (level 1); 5 mg/ml × min and 50 mg/m^2, respectively (level 2), and 5 mg/ml × min and 60 mg/m^2, respectively (level 3). Although a phase I trial using this drug combination against non-SCLC performed at our institution found that the recommended dose was level 3, as the current trial included only elderly patients, the starting dose used was level 2. However, if a patient had history of prior chemotherapy, performance status (PS) of 2, or was aged 75 years or more, the dose administered was reduced by 1 level. If a patient had a PS of 0, the dose was increased by 1 level. Cycles were repeated every 4 weeks, and patients aged 70 years or more with a PS of 0–2 were eligible.

Results: Eighteen patients were enrolled, of which nine were given the level 1 dose, seven the level 2 dose, and two the level 3 dose. The patient group had a median age of 75 years, 8 patients had limited disease (LD) versus 10 with ED, 9 had received previous treatment for SCLC versus 9 previously untreated, and 13 had a PS of 0–1 versus 5 with a PS of 2. Seventeen (94%) patients

☆ Presented in part at the 40th Annual Meeting of the American Society of Clinical Oncology, New Orleans, Louisiana, June 5–8, 2004.

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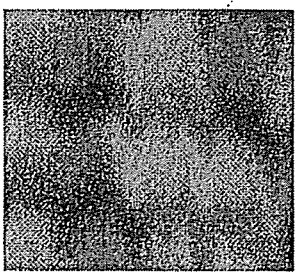
0169-5002/$ – see front matter
doi:10.1016/j.lungcan.2006.05.004



received two or more cycles of chemotherapy, and the median actual delivery of irinotecan was 84% of the projected dose. Grade 3/4 neutropenia, anemia, and diarrhea occurred in 50%, 33% and 6% of patients, respectively. Other toxicities were mild and no treatment-related deaths occurred. The response rate was 89%, with two complete responses and 14 partial responses. The median survival time was 13.3 months and the 1-year survival rate was 62%.
Conclusions: The combination of carboplatin and irinotecan with G-CSF support was an effective and non-toxic regimen in elderly SCLC patients and should be further evaluated in phase III trials.

1. Introduction

Approximately half of the patients with small cell lung cancer (SCLC) are older than 70 years, and this proportion of elderly SCLC patients is expected to increase in Japan [1–3]. Commonly used combination chemotherapy regimens for non-elderly SCLC include: cyclophosphamide, doxorubicin, and vincristine (CAV); cisplatin and etoposide (PE); alternating PE/CAV; and irinotecan plus cisplatin (IP) [4–6]. However, since many studies arbitrarily exclude elderly patients from clinical trials, no standard chemotherapeutic regimen has yet been established for elderly SCLC patients.

The Japan Clinical Oncology Group (JCOG) concluded that carboplatin plus etoposide (CE) represented an effective regimen with low toxicity in elderly SCLC patients in a phase II trial [7], and showed that IP was more effective than PE in the treatment of non-elderly patients with extensive disease (ED)-SCLC in a phase III trial [6]. As few clinical trials have evaluated the role of irinotecan in elderly patients with SCLC, we decided to conduct a prospective feasibility study designed to evaluate the efficacy of the carboplatin plus irinotecan (CI) regimen in elderly SCLC patients.

2. Patients and methods

2.1. Patient selection

Eligibility criteria were previously treated or untreated patients with histologically or cytologically confirmed SCLC, ≥70 years in age, and with an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0–2. Additional criteria were the presence of limited disease (LD) or ED (all stages of SCLC were eligible), presence of evaluable or measurable disease, expected survival ≥2 months, adequate organ function [leukocyte count ≥4000/mm^3, platelet count ≥100,000/mm^3, hemoglobin level ≥9.0 g/dl, AST/ALT ≤2 × upper limit of normal range, total bilirubin ≤1.5 mg/dl, creatinine ≤1.5 mg/dl, creatinine clearance (Ccr) ≥50 ml/min, and PaO_2 ≥60 mmHg], absence of pericardial or pleural effusions requiring drainage, absence of active concomitant malignancy, no senile dementia, and written informed consent. ED was defined as presence of distant metastases, contralateral hilar-node metastases, or pleural effusion. Exclusion criteria included brain metastases or superior vena cava (SVC) syndrome that required radiotherapy, and serious medical or psychiatric illness. Staging procedures included chest X-ray, computed tomography (CT) scan of the chest, CT scan or magnetic resonance imaging (MRI) of the brain, CT scan or ultrasound of the abdomen, and isotope bone scanning.

2.2. Treatment protocol

Treatment consisted of carboplatin administered intravenously on day 1 plus irinotecan administered intravenously on days 1, 8, and 15. Granulocyte colony-stimulating factor (G-CSF) at 50 μg/m^2 or 2 μg/kg was administered daily except on days 1, 8, 15, until leukocyte counts exceeded 10,000/mm^3, at which point the G-CSF was discontinued. If leukocyte counts decreased to less than 3000/mm^3, G-CSF treatment was restarted. Cycles were repeated every 4 weeks for up to four courses. This trial used three carboplatin area under the curve (AUC) and irinotecan dose levels of 4 mg/ml × min carboplatin and 50 mg/m^2 irinotecan (level 1), 5 mg/ml × min carboplatin and 50 mg/m^2 irinotecan (level 2), and 5 mg/ml × min carboplatin and 60 mg/m^2 irinotecan (level 3). Based on a phase I trial of combined carboplatin and irinotecan for non-SCLC performed at our institution, level 3 was determined to be the recommended dose [8]. However, as the current trial included only elderly patients, the starting dose was reduced to level 2. If a patient had history of prior chemotherapy, performance status (PS) of 2, or was 75 or more years old, the dose administered was reduced by 1 level. If a patient had a PS of 0, the dose was increased by one level. For example, if a patient had a PS of 0 and was 78-years old, the patient received level 2 dose. If a patient had a PS of 2 and was 73-years old, the patient received level 1 dose. The 24 h Ccr was substituted for glomerular filtration rate (GFR) in Calvert's formula [9]. Antiemetic prophylaxis with 5-HT_3 antagonists plus dexamethasone was routinely used. In cases of irinotecan-induced diarrhea, high dose loperamide treatment was given as described in Abigerges et al. [10]. Irinotecan was withdrawn if leukocyte counts were less than 3000/mm^3, platelet counts less than 75,000/mm^3, or if diarrhea of grade 1 or more occurred on days 8 and 15. Subsequent courses of chemotherapy were initiated when leukocyte counts reached 4000/mm^3 and platelet counts 100,000/mm^3 after day 28 and for 2 or more days after the discontinuation of G-CSF. If the above criteria were not satisfied by the first day of the next course, treatment was withheld until full recovery. If more than 6 weeks passed from the first day of the last course, the patient was taken out of the study. Dose modifications were made for both carboplatin and irinotecan based on toxicity. Patients that experienced grade 4 leukopenia or neutropenia, grade 2 diarrhea, or neutropenic fever received a 25% reduction in irinotecan dose for the next course. Patients that experienced grade 3 or 4 thrombocytopenia received a 20% reduction in target carboplatin AUC for the next course. If the same toxicity occurred following dose reduction, the patient was taken out of the study. If grade 3 or 4 non-hematologic toxici-

ties, except for nausea/vomiting and hyposodium, occurred, the patient was taken out of the study even if the toxicities improved thereafter. Patients with LD received thoracic irradiation after chemotherapy. Palliative radiotherapy of less than 20 Gy total dose was allowable to control persistent pain associated with bone metastasis during the study period. After the completion of four courses, responders did not receive further chemotherapy unless progressive disease (PD) developed. Post-protocol treatments were left at the discretion of the physician. Prophylactic cranial irradiation (PCI) was an option for patients that achieved a complete response (CR).

2.3. Evaluation

Tumor responses were evaluated according to World Health Organization criteria [11]. A CR was defined as the complete disappearance of tumor for at least 4 weeks. A partial response (PR) was defined as a ≥50% reduction in the sum of the products of the two greatest perpendicular diameters of all indicator lesions or a reduction of more than 50% in assessable disease for at least 4 weeks, with no appearance of new lesions or progression of any existing lesions. PD was defined as a ≥25% increase in tumor area or the appearance of new lesions. All other outcomes were classified as no change (NC). At the time of study initiation in March 1998, response evaluation criteria in solid tumors (RECIST) [12] was not yet available, such that toxicities were evaluated according to the National Cancer Institute-Common Toxicity Criteria (NCI-CTC).

2.4. Study design and statistics

This trial was designed as a prospective non-phase I study and the main objective is to see feasibility and efficacy. The study protocol was approved by the institutional review board at our institution prior to the initiation of the study. Study objectives were to detect and quantify the clinical toxicities of the carboplatin and irinotecan combination and to assess its therapeutic efficacy in elderly patients with SCLC. Because this feasibility study included a heterogeneous patient population, (e.g. in terms of presence of prior chemotherapy and disease stage), the study was not designed as a phase I or II study. Therefore, sample size calculations based on Simon's minimax design were not applied to this study. Analysis of the trial was based on the intention-to-treat principle. Overall survival, determined from the time of registration to death or the last follow-up evaluation, was calculated using the Kaplan and Meier method.

3. Results

3.1. Patient characteristics

Between March 1998 and December 2003, 18 patients were registered for the study, and all received chemotherapy. Patient characteristics are listed in Table 1. Patients consisted of 4 women and 14 men, with a median age of 75 years (range, 70–85 years) and a median 24 h Ccr of 74 ml/min (range, 28–134 ml/min). Thirteen patients

Table 1 Patient characteristics

No. of patients	18
Male/female	14/4
Median age, years (range)	75 (70–85)
Stage: LD/ED	8/10
PS (ECOG): 0/1/2	4/9/5
Prior chemotherapy: present/absent	9/9
Sensitive/refractory cases	5/4
Median 24 h Ccr, ml/min (range)	74 (28–134)

LD, limited disease; ED, extensive disease; PS, performance status; ECOG, Eastern Cooperative Oncology Group; Ccr, creatinine clearance.

Table 2 Dose level

Level	No. of patients	AUC of carboplatin (mg/ml × min)	Dose of irinotecan (mg/m²)
1	4	4	50
2	7	5	50
3	2	5	60

AUC, area under the curve.

(72%) had an ECOG PS of 0 or 1. Eight patients had LD and 10 had ED. Nine patients had a history of prior chemotherapy (five with sensitive relapses, four with refractory relapses) and nine were chemo-naïve. Of the previously treated patients, five had received one regimen of CE and two had received one regimen of PE. One patient had received two regimens consisting of CE and CODE (cisplatin + oncovin + doxorubicin + etoposide), and one patient had received three regimens consisting of CE, CODE and IP. The numbers of patients that started at dose levels 1, 2 and 3 were nine, seven and two, respectively (Table 2).

3.2. Treatment delivery

Nine patients (50%) received four courses of treatment, two (11%) received three courses, six (33%) received two courses, and one (6%) received one course. The reasons for termination of treatment included completion of two or more courses of chemotherapy (16 patients, 89%), and NC (two patients, 11%). One patient experienced grade 3 diarrhea after receiving a single course of chemotherapy and was taken off the study. No treatment-related deaths (TRDs) occurred. Course intervals and dose reductions are listed in Table 3. The median interval of each round of chemotherapy was 28–29 days. Only four patients received a reduced dose

Table 3 Course interval and dose reduction

Interval of each chemotherapy course	No. of patients	Median days (range)	No. of patients with dose reduction
1–2	17	28 (21–35)	4
2–3	10	29 (25–36)	0
3–4	9	28 (27–35)	0

Thrombocytopenia, two patients; neutropenia, one patient; both, one patient.

Table 4 Hematologic toxicity (worst of any course)

Level	No. of patients	Leukopenia			Neutropenia			Anemia			Thrombocytopenia		
		Grade 2	Grade 3	Grade 4	Grade 2	Grade 3	Grade 4	Grade 2	Grade 3	Grade 4	Grade 2	Grade 3	Grade 4
1	5	5	0	0	2	4	0	5	3	0	2	0	0
2	7	2	3	0	1	3	1	2	3	0	2	1	2
3	2	1	1	0	1	1	0	2	0	0	0	0	0

Table 5 Non-hematologic toxicity (worst of any course)

Level	No. of patients	Nausea/vomiting				Diarrhea				Infection			Pulmonary		Liver		Renal	
		Grade 1	Grade 2	Grade 3	Grade 4	Grade 1	Grade 2	Grade 3	Grade 4	Grade 1	Grade 2	Grade ≥ 3	Grade 2	Grade ≥ 3	Grade 1	Grade ≥ 2	Grade 2	Grade ≥ 3
1	5	5	0	1	0	3	1	1	0	1	1	0	0	0	1	0	0	0
2	7	7	0	0	0	3	2	0	0	0	0	0	0	0	1	0	0	0
3	2	2	0	0	0	1	0	0	0	1	0	0	0	0	1	0	0	0

Table 6 Therapeutic response

Level	No. of patients	Previously treated patients (refractory)	Response			
			CR	PR	NC	PD
1	9	6 (2)	1	6	2	0
2	7	3 (1)	1	6	0	0
3	2	0	0	2	0	0

Overall response rate (ORR) = 16/18 (89%); RR for previously untreated patients = 9/9 (100%); RR for previously treated patients = 7/9 (78%). CR, complete response; PR, partial response; NC, no change; PD, progressive disease.

in the second course due to toxicities experienced during the first course. The reasons for dose reduction were thrombocytopenia in two patients, neutropenia in one patient and both thrombocytopenia and neutropenia in one patient. However, no patients experienced further toxicities after dose reduction. Median percentage of irinotecan dose intensity (mg/m^2/week), expressed as the actual delivered dose as a percentage of the projected dose, was 84% (range: 48–100%). Of the 162 projected irinotecan infusions, 18 dose omissions occurred during the study period due to leukopenia in five cases, thrombocytopenia in four cases, diarrhea in eight cases and patient refusal in one case. Therefore, the percentage of actual irinotecan infusions, based on actually delivered infusions as a percentage of projected infusions, was 89% (144/162).

3.3. Toxicity

Hematologic and non-hematologic toxicities are listed in Tables 4 and 5. Grade 3 or 4 neutropenia, anemia, and thrombocytopenia occurred in 50%, 33%, and 17% of patients, respectively. However, neither grade 4 leukopenia nor anemia occurred at all three dose levels. Non-hematologic toxicities were generally mild, and grade 3 diarrhea and grade 3 nausea/vomiting occurred in only one patient each. Other non-hematologic toxicities were also mild, and no grade 3 or 4 toxicities except for gastrointestinal toxicities occurred at all three dose levels.

3.4. Response and survival

Chemotherapeutic responses are listed in Table 6. Of the 18 patients, two showed CRs and 14 PRs, giving a response rate of 89% (16/18). For the nine chemo-naïve patients, the response rate was 100% (9/9). In contrast, of the nine previously-treated patients, seven responded to treatment, giving a response rate of 78% (7/9). Of the four patients with refractory relapses, two responded. The median survival time (MST) and 1-year survival rate for all 18 patients in the study was 13.3 months and 62%, respectively (Fig. 1).

4. Discussion

Until recently, there was no standard chemotherapeutic regimen for elderly SCLC patients. However, four comparative studies, including two phase III [13,14] and two randomized phase II [15,16] trials, have shown that suboptimal chemotherapies, such as oral etoposide monotherapy or attenuated doses of combination chemotherapy, may lead to reduced survival in elderly or poor-risk SCLC patients when compared with standard doses of combination chemotherapies.

To our knowledge, this is the first study to evaluate the CI regimen in elderly patients with SCLC. The response rate of the CI regimen was 89%, with an MST of 13.3 months. These were very promising results, especially as this study included only elderly SCLC patients and half of the study group had already received some form of chemotherapy, although this study included both ED and LD patients as the same population. Observed instances of toxicity tended to be mild and no TRDs occurred. Although a near full-dose combination chemotherapy was administered to the elderly SCLC patients in our study, only half of the patients experienced grade 3/4 neutropenia. Furthermore, the irinotecan dose intensity of 84% was relatively high. It is possible that the acceptable toxicities and dose intensity were largely attributable to the prophylactic use of G-CSF and the high-dose loperamide therapy against irinotecan-induced diarrhea. On the other hand, other phase I studies, which also included patients over the age of 70, demonstrated that carboplatin AUC 5 and irinotecan 50 mg/m^2 can be safely administered without G-CSF prophylaxis [17–19]. However, these studies were not specifically designed to the elderly population and the median age of these studies were clearly younger than that of our trial.

Several retrospective analyses [20–22] and a prospective study [23] have shown that standard-dose chemotherapy without G-CSF support can lead to an increased risk of early death and sepsis in older populations. Moreover, American

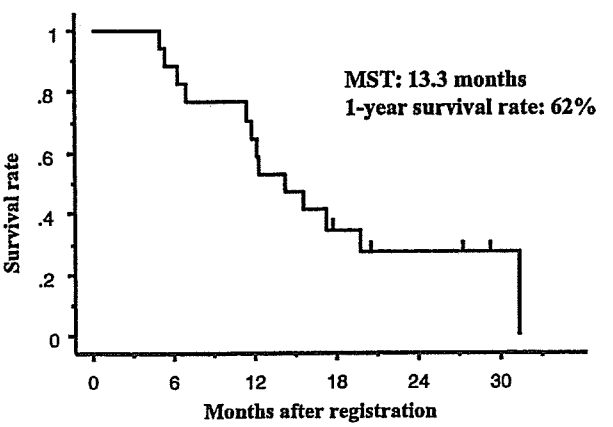


Fig. 1 Overall survival curve.