Treatment of lung damage

Retrospective analysis of steroid therapy for radiation-induced lung injury in lung cancer patients

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Abstract

Purpose: To disclose characteristics of lung cancer patients developing radiation-induced lung injury treated with or without corticosteroid therapy.

Methods and materials: Radiographic changes, symptoms, history of corticosteroid prescription, and clinical course after 50—70 Gy of thoracic radiotherapy were retrospectively evaluated in 385 lung cancer patients.

Results: Radiation-induced lung injury was stable without corticosteroid in 307 patients (Group 1), stable with corticosteroid in 64 patients (Group 2), and progressive to death despite corticosteroid in 14 patients (Group 3). Fever and dyspnea were noted in 11%, 50% and 86% ($p < 0.001$), and in 13%, 44% and 57% ($p < 0.001$) patients in Groups 1—3, respectively. Median weeks between the end of radiotherapy and the first radiographic change were 9.9, 6.7 and 2.4 for Groups 1—3, respectively ($p < 0.001$). The initial prednisolone equivalent dose was 30—40 mg daily in 52 (67%) patients. A total of 16 (4.2%) patients died of radiation pneumonitis or steroid complication with a median survival of 45 (range, 8—107) days.

Conclusion: Development of fever and dyspnea, and short interval between the end of radiotherapy and the first radiographic change were associated with fatal radiation-induced lung injury. Prednisolone 30—40 mg daily was selected for the treatment in many patients.
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Keywords: Radiation pneumonitis; Radiotherapy; Lung cancer; Corticosteroid

Thoracic radiotherapy is widely used for the curative and palliative treatment of lung cancer. Radiation-induced lung injury was first described as early as 1922 [1,2], and two types of lung injury, radiation pneumonitis and radiation fibrosis, were recognized in 1925 [3]. Radiation pneumonitis occurs in 5—15% of patients who have received radiation therapy for lung cancer. Its clinical symptoms are characterized by cough, dyspnea and fever developing between 1 and 3 months after the end of radiotherapy. Distinctive radiographic changes of radiation pneumonitis are a ground-glass opacification or diffuse haziness in early phase, and then alveolar infiltrates or dense consolidation in late phase in the region corresponding to the irradiated area [4—7]. Radiation pneumonitis may persist for a month or more and subside gradually. In severe cases, however, pneumonitis progresses to death due to respiratory failure within few weeks [4].

Use of adrenocorticotropic hormone (ACTH) and cortisone for radiation pneumonitis in a case was first reported in 1951 [8], and 9 cases of radiation pneumonitis treated with cortisone therapy in the literature were reviewed in 1968 [9]. Although no case series or clinical trials of corticosteroid therapy have been reported since that time, prednisolone has been given in patients with severe pneumonitis in clinical practice. The initial dose of prednisolone, approximately 30—100 mg daily, and very slow tapering schedule are in agreement among experts [4—6,10], because early withdrawal results in aggravation of pneumonitis [11—13]. There is no consensus, however, about criteria to define when steroids are required for radiation-induced lung injury. The objective of this study is to disclose general characteristics of lung cancer patients developing radiation-induced lung injury treated with or without corticosteroid therapy, to obtain data on the initiation criteria, dose, and taper schedule of corticosteroid therapy for further prospective trials.

Patients and methods

Consecutive lung cancer patients treated with thoracic radiotherapy at a total dose of 50—70 Gy in National Cancer

Center Hospital between January 1998 and December 2003 were subjects of this study. We retrospectively reviewed all chest X-ray films taken during 6 month period from the end of thoracic radiation to identify the first radiographic change and its progress. History of corticosteroid prescription, symptoms at the time of and one-month period after the first radiographic change in a chest X-ray film, and clinical course of radiation-induced lung injury were obtained from medical charts. The diagnosis of radiation-induced lung injury was defined as radiographic changes including opacification, diffuse haziness, infiltrates or consolidation conforming to the outline of the sharply demarcated irradiated area in a chest X-ray film. During clinical course, scarring (fibrosis) was developed within the irradiated area leading to a reduction in lung volume. In contrast, pulmonary infection spreads through anatomical structure of the lung, and the boundary of infiltrates corresponds to anatomical boundary of the lung. For patients with fever, the radiographical response to antibiotics was also evaluated. Observed differences in the proportions of patients in various patient subgroups were evaluated using Chi-square test. Differences between continuous variables were compared using Mann—Whitney tests. The Dr. SPSS II 11.0 for Windows software package (SPSS Japan Inc., Tokyo, Japan) was used for all statistical analyses.

Results

Of 544 lung cancer patients receiving thoracic radiotherapy at a total dose of 50—70 Gy, 111 patients were excluded from this study because they were not evaluable: loss of follow-up in 88 patients, early lung cancer progression in 18 patients, chemotherapy-induced neutropenic fever and pneumonia in three patients, death of bleeding from the esophageal stent in one patient, and no chest X-ray films available in one patient. In addition, 48 patients (11% of 433 evaluable patients) were also excluded because no radi-

ation-induced lung injury was noted. Thus, the subject of this study was 385 patients.

Of the 385 patients, 78 (20%) received corticosteroid therapy for radiation-induced lung injury, and 307 did not. Radiation-induced lung injury was stable without corticosteroid in the 307 (80%) patients (Group 1), stable or in remission with corticosteroid in 64 (17%) patients (Group 2), and progressive to death despite corticosteroid in 14 (4%) patients (Group 3). No difference in sex, total dose, intent of radiotherapy, and combination chemotherapy was noted among three Groups, but median age of patients was higher in Group 3 (Table 1). Fever was developed in 50% of patients in Group 3 at the initial radiographic change, and in 86% of them during subsequent clinical course, while it was developed in only 11—12% of patients in Group 1 through their clinical course (Table 2). Dyspnea was developed in 57% of patients in Group 3 and in 44% of patients in Group 2 during clinical course, while it was developed in only 14% of patients in Group 1 (Table 2). A total of 88 patients developed fever at the initial change in chest X-ray and/or during subsequent clinical course. Of these, 43 patients received antibiotics, but no radiographical response was obtained in these patients. Five (2%) and seven (2%) patients in Group 1 developed bloody sputum and chest pain, respectively, but none in Group 2 or 3 developed these symptoms. The average interval of chest X-rays taken between the start of radiotherapy and the first appearance of radiographic change was 1.7 weeks for group 1, 1.3 weeks for group 2, and 0.9 weeks for group 3 ($P < 0.001$, Table 3). Interval between the end of radiotherapy and the first change in a chest X-ray was shorter in Group 3 than in Group 2 or Group 1 (Table 3). Of 57 patients in whom the first radiographic change was noted within three weeks, 9 (16%) died of pneumonitis, while radiation-induced lung injury that occurred 10 weeks or later after the end of radiation was easily managed with or without steroid therapy (Table 3). Oxygen content in the blood at the start of steroid therapy was examined in 70 patients of Groups 2 and 3. Oxygen content

Table 1
Patient demographics and radiotherapy performance

Characteristics	Total N (%)	Group 1 N (%)	Group 2 N (%)	Group 3 N (%)	p-value
Total	385 (100)	307 (80)	64 (17)	14 (4)	
Sex					
Male	300 (78)	240 (78)	47 (73)	13 (93)	0.28
Female	85 (22)	67 (22)	17 (27)	1 (7)	
Age median (range)	65 (28—87)	63 (28—87)	65 (37—83)	71 (65—84)	0.008
Total dose (Gy)					
Median (range)	60 (50—70)	60 (50—70)	60 (50—61)	60 (50—60)	0.50
Intent of radiotherapy					
Curative	298 (77)	232 (76)	52 (81)	14 (100)	0.074
Palliative	87 (23)	75 (24)	12 (19)	0 (0)	
Chemotherapy					
None	121 (31)	101 (33)	15 (23)	5 (36)	0.48
Sequential	121 (31)	93 (30)	25 (39)	3 (21)	
Concurrent	143 (37)	113 (37)	24 (38)	6 (43)	

Table 2
Symptoms through clinical courses

Symptom	At the initial change in chest X-ray				During subsequent clinical course			
	Group 1	Group 2	Group 3	p	Group 1[a]	Group 2[b]	Group3[b]	p
Cough	96 (31)	35 (56)	5 (36)	0.001	85 (28)	38 (59)	5 (36)	<0.001
Sputum	32 (10)	11 (18)	4 (29)	0.049	30 (10)	11 (17)	3 (21)	0.12
Hemosputum	5 (2)	0 (0)	0 (0)	0.53	4 (1)	0 (0)	0 (0)	0.60
Chest pain	7 (2)	0 (0)	0 (0)	0.40	2 (0.6)	0 (0)	0 (0)	0.78
Fever								
None	269 (88)	35 (56)	7 (50)	<0.001	272 (89)	32 (50)	2 (14)	<0.001
37.0–37.9 °C	18 (6)	11 (18)	2 (14)	24 (8)	16 (25)	5 (35)		
38 °C≤	13 (4)	14 (22)	5 (36)	8 (3)	13 (20)	7 (50)		
Not specified	7 (2)	3 (4)	0 (0)	3 (1)	3 (4)	0 (0)		
Dyspnea	43 (14)	14 (22)	6 (43)	0.007	40 (13)	28 (44)	8 (57)	<0.001
Fever or dyspnea	75 (24)	37 (58)	10 (71)	<0.001	65 (21)	49 (77)	14 (100)	<0.001
Any	150 (49)	51 (81)	13 (93)	<0.001	118 (38)	60 (94)	14 (100)	<0.001

[a] During one month period following the initial change in the chest X-ray.
[b] At the start of steroid therapy.

Table 3
The chest X-ray intervals and first radiographic change

Weeks	Group 1	Group 2	Group 3	p-value
The average interval of chest X-rays (weeks)[a]				
Median (range)	1.7 (0.7 to 6.0)	1.3 (0.5 to 4.4)	0.9 (0.5 to 3.8)	<0.001
Duration between the end of radiotherapy and the first radiographic change (weeks)				
Median (range)	9.9 (−2.9 to 45.1)	6.7 (0 to 24.9)	2.4 (0.4 to 10.1)	<0.001
<6	82 (27)	26 (41)	11 (79)	<0.001
6–11.9	116 (38)	29 (45)	3 (21)	
12–17.9	71 (23)	7 (11)	0 (0)	
18≤	38 (12)	2 (3)	0 (0)	

[a] Calculated as follows: the average interval of chest X-rays = (the first radiographic change − the start of radiotherapy)/the number of chest X-rays taken during this period/7).

was slightly decreased (PaO2 = 70–74.9 Torr) in 12 (19%) patients of Group 2 and one (7%) patient of Group 3, and moderately to severely decreased (PaO2 ⩽ 69.9 Torr or SpO2 ⩽ 92%) in 21 (33%) patients of Group 2 and 7 (50%) patients of Group 3 (p = 0.38).

Prednisolone was administered as the initial therapy in 69 (88%) patients of Groups 2 and 3. The initial prednisolone equivalent dose of steroid was 30–40 mg daily in 52 (67%), and 60 mg of higher only in 8 (10%) patients (Table 4). The median duration of the initial dose was 10 (range, 2–64) days, and the dose was reduced within 14 days in 57 (77%) patients. The median duration of steroid therapy was 10 (range, 2–28) weeks (Table 4). Steroid pulse therapy (methylprednisolone 1000 mg daily for three days) was administered as the initial therapy in one patient, and as salvage therapy in six patients at the time of pneumonitis aggravation. Among the seven patients, six died of respiratory failure due to progressive radiation pneumonitis.

Outcome of steroid therapy was evaluated in 76 patients (Fig. 1). Symptomatic relief was obtained and the steroid dose was reduced in 71 (93%) of the 76 patients, while no effect was noted in the remaining five patients, who all died of radiation pneumonitis despite escalated steroid administration. Of the 71 patients, 15 (21%) developed recurrent symptoms at the median daily prednisolone dose of 20 mg

(range, 10–40 mg) within median 33 days (range, 21–42 days) from the start of the steroid therapy, and required steroids to be escalated. Of the 15 patients, nine died of radiation pneumonitis and one died of complication of steroid therapy. A total of 54 (71%) patients were in remission from pneumonitis and steroid therapy was terminated. The remainder 22 patients died during steroid therapy, 14 of radiation pneumonitis, two of infectious complication (bacterial pneumonia in one, and lung aspergillosis in another patient), five of lung cancer progression, and one of hemoptysis. Thus, 16 patients, who accounted for 4.2% of 385 patients receiving 50–70 Gy of thoracic radiotherapy, and who accounted for 21% of 78 patients treated with steroid therapy, died of radiation pneumonitis or complication associated with steroid therapy. Median survival from the start of steroid therapy in these patients was 45 (range, 8–107) days.

Discussion

Patients with radiation-induced lung injury have been managed in compliance with the expert opinions, because there has been no case series or clinical trial report on clinical course and corticosteroid use for this lung injury. This

Table 4
Corticosteroid, dose and duration of steroid therapy

	N (%)
Corticosteroid	
Prednisolone	69 (88)
Dexamethasone	4 (5)
Betamethasone	4 (5)
Methylprednisolone	1 (1)
Initial dose, mg/body daily (prednisolone equivalent)	
Pulse therapy	1 (1)
60	7 (9)
50	1 (1)
40	10 (13)
30	42 (54)
10–25	17 (22)
Duration of the initial dose, days	
Median (range)	10 (2–64)
≤14	57 (77)
15–28	9 (12)
29≤	8 (11)
Not evaluable	4
Total duration of steroid therapy, weeks	
Median (range)	10 (2–28)
≤6	16 (30)
6.1–12	19 (35)
12.1–18	14 (26)
18.1≤	5 (9)
Not evaluable	24

study is the first systemic review of these patients both who received corticosteroid therapy and who did not. Comparison between the expert opinions and the results of this study is given below. First, radiation-induced lung injury is severer when a radiographic change appears earlier [5]. In

this study, the initial change in a chest X-ray film was observed in 9.9 (range, −3 to 45) weeks in Group 1, in 6.7 (range, 0–25) weeks in Group 2, and 2.4 (range, 0–10) weeks in Group 3 after the end of thoracic radiotherapy. If patients present with symptoms, presumably they receive a chest X-ray. Thus, the patients with symptoms may have radiographic findings seen sooner, since they receive an X-ray when they complain of symptoms. The average interval of chest X-rays taken between the start of radiotherapy and the first appearance of radiographic change was longer in Group 1 than that in groups 2 and 3. The difference, however, was negligibly small when compared with the difference in duration between the end of radiotherapy and the first radiographic change. Second, steroid administration is determined generally based on the severity of symptoms [5]. In this study steroid was used when patients developed dyspnea or fever. Dyspnea has been thought to be the cardinal symptom of radiation pneumonitis but fever to be unusual [5,10]. In this study, however, fever was highly associated with fatal radiation pneumonitis; fever was noted in 12% patients of Group 1, in 58% patients of Group 2, and 86% patients of Group 3. This study failed to show utility of blood gas analysis. An oxygen content in the blood was decreased moderately to severely in only 28 (36%) patients in Groups 2 and 3, and did not differ between the two groups. The oxygen content in Group 1 was measured in only small number of patients, and therefore it was not evaluable in this study. Third, 30–100 mg/day of prednisolone has been recommended as the initial dose [4–6,10]. In our practice, a dose of 30–40 mg was the most frequently used. We selected this relatively low dose of steroid mostly because steroid therapy was started in out patient clinic. Forth, duration of the initial dose was within two weeks in 73% of patients, which is consistent to most expert opinions [6,10]. In contrast, tapering schedules varied between a pa-
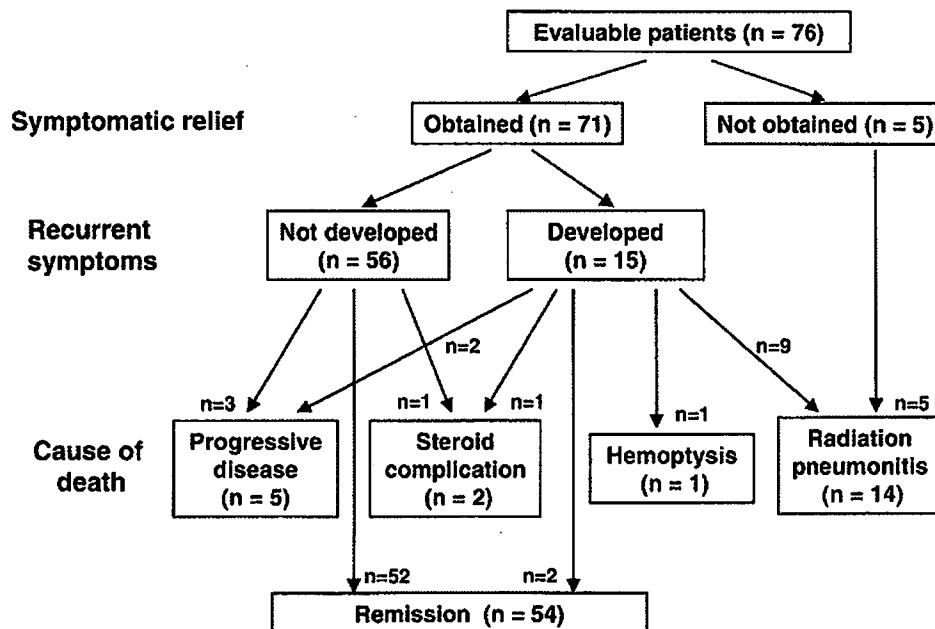

Fig. 1. Outcome of patients who received steroid therapy. Two patients were excluded because of loss of follow-up. Of 76 evaluable patients, 71 (93%) experienced symptomatic relief by steroid therapy.

tient and another in this study. This may be partly due to the diversity in clinical course of radiation pneumonitis, but mostly due to lacking in available recommendation for tapering schedules. In this study, median total duration of steroid therapy was 10 weeks, which may be a tentative guide. A guideline of taper schedule appeared in the latest textbook: the dose should be tapered by 10 mg every two weeks, and be terminated in 12 weeks [10].

Although our clinical practice mostly followed the expert opinions on the management of radiation-induced lung injury as mentioned above, there is little evidence that our steroid use, dose and duration for radiation-induced lung injury were correct. In this study, 21% of patients received steroid therapy and 4% of patients died of radiation pneumonitis among lung cancer patients treated with thoracic radiotherapy at a total dose of 50 Gy or higher. These figures are comparable to the incidence of grade 3 pneumonitis, 3—20%, and that of fatal pneumonitis, 1—4%, in other reports [10].

In conclusion, development of fever and dyspnea, and short interval between the end of radiotherapy and the first radiographic change were associated with fatal radiation-induced lung injury. Prednisolone 30—40 mg daily for two weeks followed by slow taper was selected for the treatment in many patients.

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

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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/m² 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-defi-

cient 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
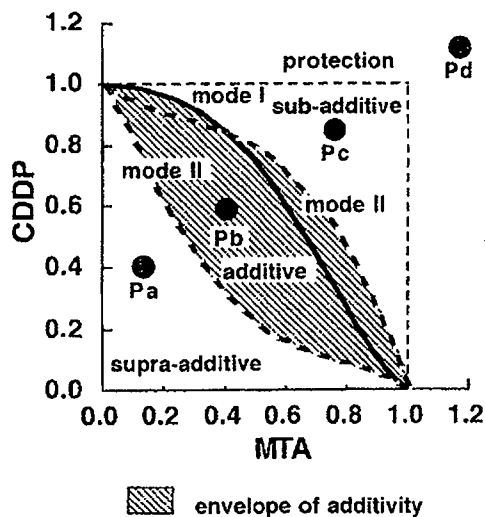

Figure 1. Schematic representation of an isobologram (29). The envelope of additivity, surrounded by mode I (solid line) and mode II (dotted lines) isobologram lines, was constructed from the dose–response curves of pemetrexed (MTA) and cisplatin (CDDP). The concentrations that produced 80% cell growth 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.
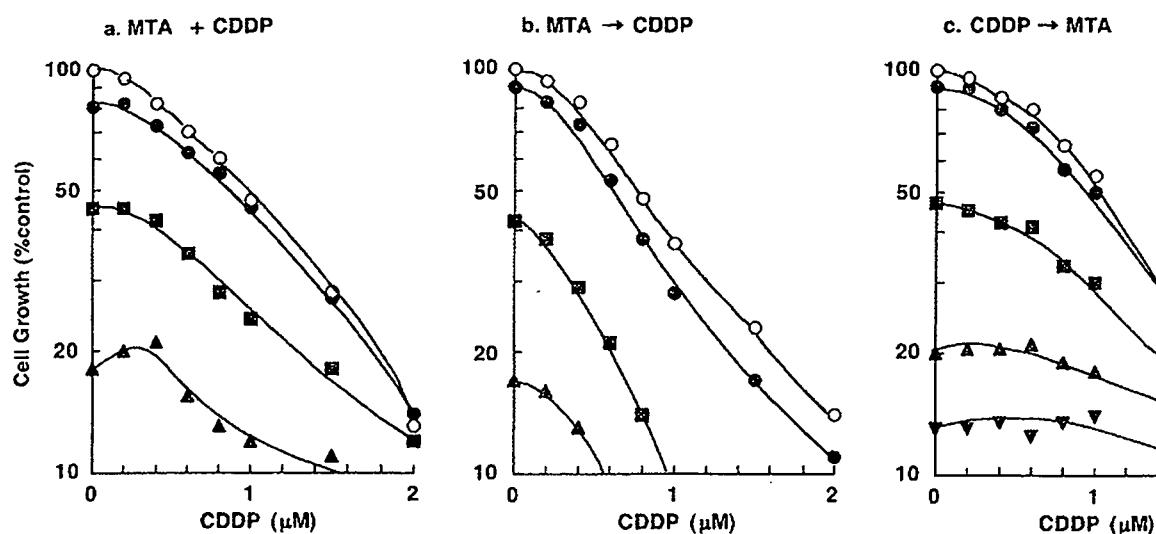
Figure 2. Schedule dependence of the interaction between pemetrexed and cisplatin in PA1 cells. Cells were exposed to these two drugs simultaneously for 24 h (a), pemetrexed first for 24 h followed by cisplatin for 24 h (b), or the reverse sequence (c). The cell number after 5 days was measured using 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-di-methylthiazol-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 che-

motherapy, 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).
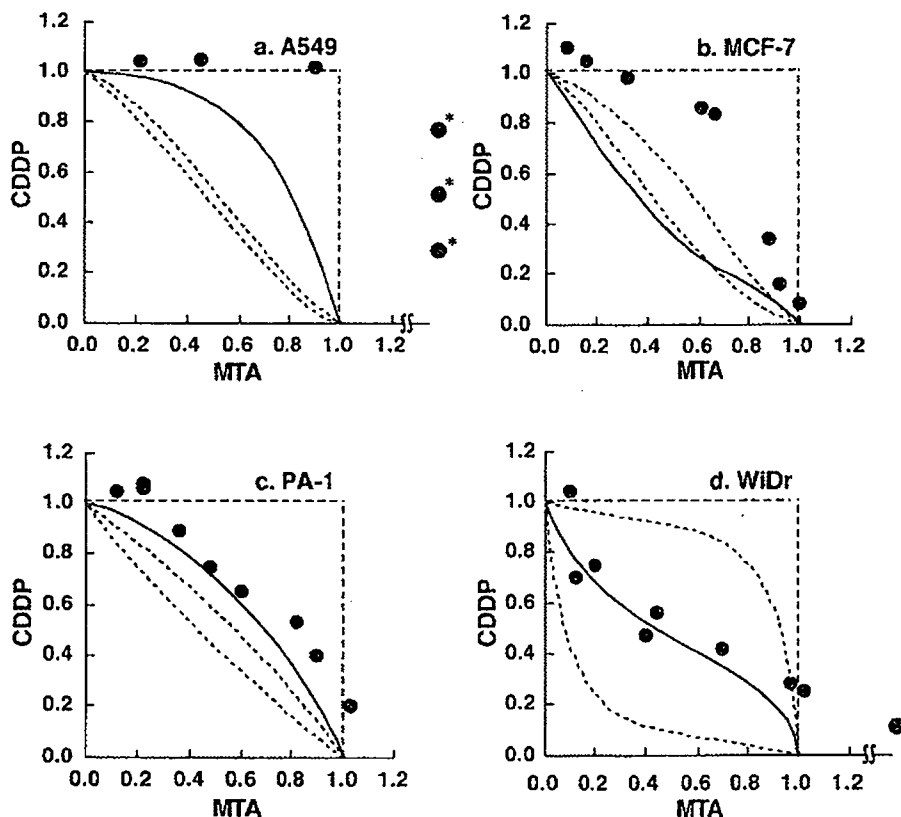

Figure 3. Isobolograms of simultaneous exposure to pemetrexed and cisplatin for 24 h in A549 (a), MCF7 (b), PA1 (c), and WiDr (d) cells. For the A549, MCF7, and PA1 cells, the combined data points fell in the areas of subadditivity and protection. For the WiDr cells, the combined data points fell mainly within the envelope of additivity. Data are mean values for at least three independent experiments; SE was <25% (*except the data).
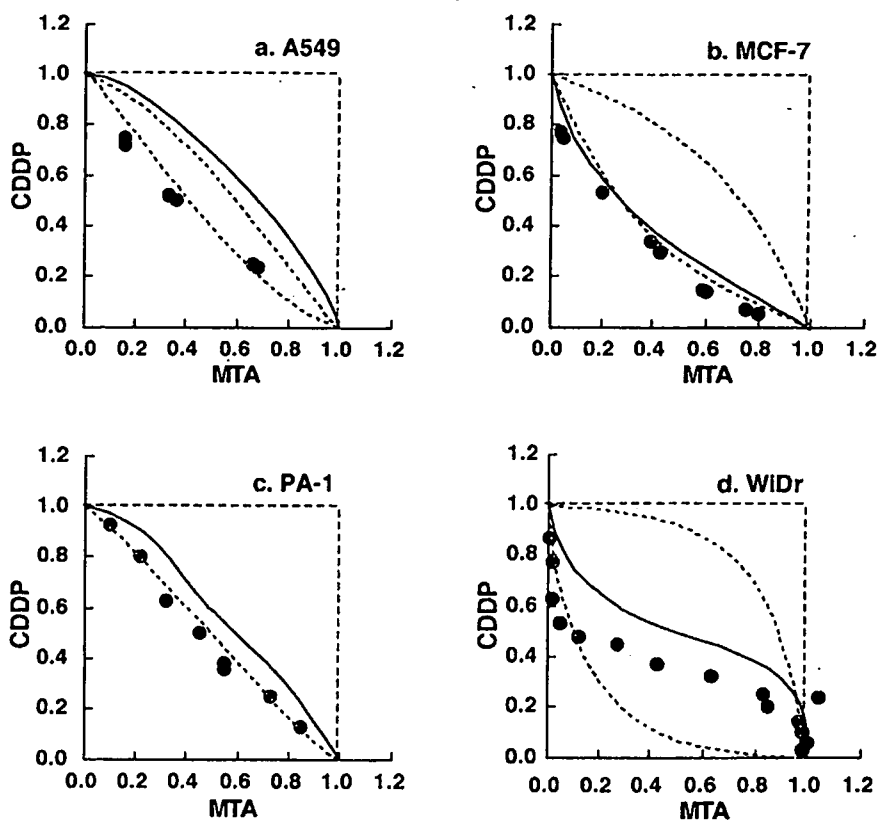
Figure 4. Isobolograms of sequential exposure to pemetrexed (24 h) followed by cisplatin (24 h) in A549 (a), MCF7 (b), PA1 (c), and WiDr (d) cells. For the A549, MCF7, and PA1 cells, all or most of the data points of the combinations fell within the envelope of additivity and in the area of supra-additivity. 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. Iso-

bolograms 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-
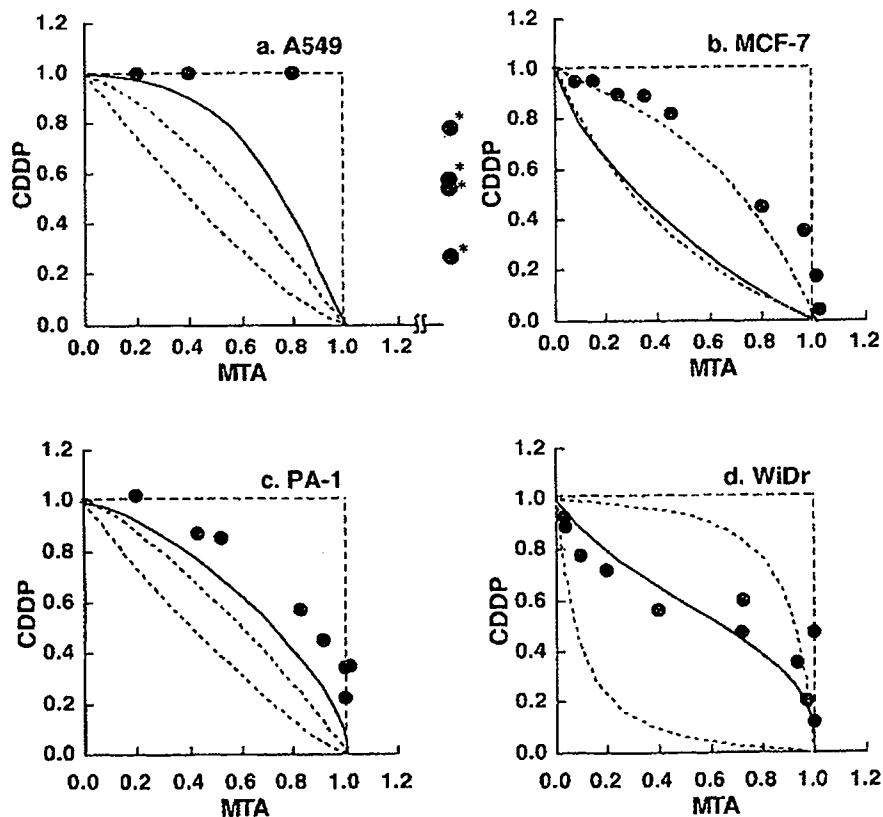
Figure 5. Isobolograms of sequential exposure to cisplatin (24 h) followed by pemetrexed (24 h) in A549 (a), MCF7 (b), PA1 (c), and WiDr (d) cells. For the A549, MCF7, and PA1 cells, all or most of the data points of the combinations fell in the areas of subadditivity and protection. For the WiDr cells, 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
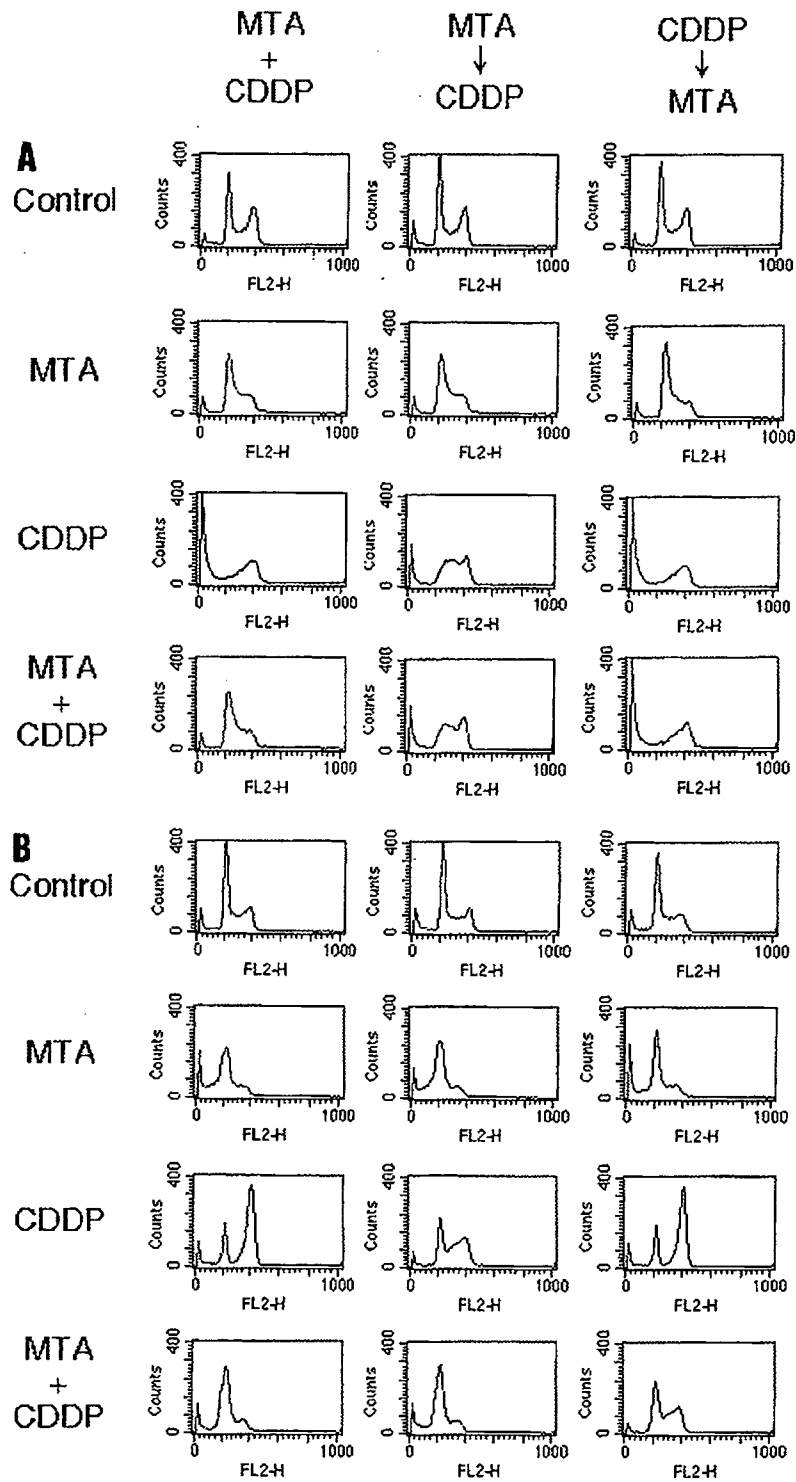
Figure 6. Flow cytometric analysis of cell cycle perturbation. PA1 cells, treated with 0.2 μM pemetrexed (MTA), 0.5 μM cisplatin (CDDP), both drugs simultaneously for 24 h, pemetrexed for 24 h followed by cisplatin for 24 h, or the reverse sequence were harvested at 48 h (A), and MCF7 cells, treated 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

Cell Cycle (%)	MTA + CDDP (24 h)				MTA (24 h) → CDDP (24 h)				CDDP (24 h)→ MTA (24 h)			
	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 pem-

etrexed 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.

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Second Primary Cancers in Patients with Stage III Non-Small Cell Lung Cancer Successfully Treated with Chemo-radiotherapy

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Background: Patients successfully treated for non-small cell lung cancer (NSCLC) remain at risk for developing second primary cancer (SPC). The purpose of the current study is to assess the incidence of SPC and the impact of smoking status on the SPC in long-term survivors with stage III NSCLC after chemo-radiotherapy.

Methods: Using the database from the Japan National Hospital Lung Cancer Study Group between 1985 and 1995, information was obtained on 62 patients who were more than 3 years disease-free survivors. Details of clinical information and most smoking history were available from the questionnaire.

Results: Nine of the 62 patients developed SPC 3.9–12.2 years (median, 6.2 years) after the initiation of the treatment. The site of SPC was 2 lung, 1 esophagus, 2 stomach, 1 colon, 1 breast, 1 skin and 1 leukemia. Among these nine, three cancers occurred inside the radiation field. The relative risk of any SPC was 2.8 [95% confidence interval (CI) 1.3–5.3]. The risk changed with the passage of time and it increased significantly (5.2 times at or beyond 7 years) after the treatment. In univariate analysis, the patients who were male, had more cumulative smoking and continued smoking, had an increased risk of SPC [relative risk (RR) 2.7, CI 1.1–5.3; RR 3.0, CI 1.2–6.2; RR 5.2, CI 1.6–11.7, respectively]. In multivariate analysis, factors including smoking status and histological type had no effect on the development of a SPC.

Conclusion: The patients with stage III NSCLC successfully treated with chemo-radiotherapy were at risk for developing SPC and this risk increased with time.

Key words: second primary cancer – non-small cell lung cancer – chemo-radiotherapy

INTRODUCTION

The introduction of combined modality therapy as chest radiotherapy (RT) and chemotherapy for patients with stage III non-small cell lung cancer (NSCLC) has resulted in achieving ~15% long time survivors (123). However, patients successfully treated for NSCLC as well as small cell lung cancer (SCLC) remain at risk for developing second primary cancer (SPC) (4). The risk of SPC in patients with NSCLC has been studied mainly in cohorts of surgically resected patients for stage I NSCLC (567). These reports suggest that the risk of developing SPC and second primary lung cancer (SPLC) is

1–4% and 1–2% per patient per year, respectively, and it appears to increase with the passage of time. Another study including stages I and II patients treated with chest RT confirmed a similar trend that the risk of developing SPC and SPLC is 4.3 and 1.4% per patient per year, respectively (8). Unlike the studies of the patients with SCLC (9–11), these did not provide adequate follow-up information to determine relative risk. Also, there has been no report to date to evaluate the risk of SPC associated with the treatment of RT with chemotherapy as well as smoking status in stage III NSCLC patients.

PATIENTS AND METHODS

Information was obtained on 1643 patients with stage III NSCLC between 1985 and 1995, using the database from the National Hospital Study Group for Lung Cancer, including

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National Hospital Organization Kinki-chuo Chest Medical Center, National Hospital Organization Toneyama Hospital and National Hospital Organization Okinawa Hospital. Among them, 547 patients were treated with chemo-radiotherapy with or without surgery. Of the 547, the 62 patients were more than 3 years disease-free survivors. The patients who relapsed within the 3 years were excluded in this study. Details of clinical information after the treatment and smoking history of the patients were obtained by a questionnaire, which was completed by directly interviewing the patients or the relatives of deceased patients, or by checking the patient's medical records.

Smoking cessation was defined as completely stopping smoking within 6 months after initiation of treatment. Smoking-related cancers include cancer of the lung, larynx and oral cavity, including pharynx, esophagus, pancreas, bladder, kidney, stomach and uterine cervix. A second primary lung cancer was diagnosed according to the criteria provided by Martini and Melamed in 1975 (12). The period of the study was taken as starting from the first day of therapy, and the date of second cancer was taken as the day of histological or cytological documentation of cancer.

For estimation of the expected values of SPC development, the period of risk began 3 years after initiation of treatment and ended with the date of death, date of last follow-up or date of diagnosis of a SPC, whichever occurred first. Age, gender and period-specific rates for cancer incidence within the period 1985-98 obtained from the Research Group for Population-based Cancer Registration in Japan were applied to the appropriate person-years of observation (13). Statistical methods for risk estimation were based on the assumption that observed number of second cancers followed a Poisson distribution (14). To calculate excess risks per 10 000 patients per year in sub-groups with significant relative risks, the expected number of cases was subtracted from the number observed. The difference was divided by person-years of observation, and multiplied by 10 000. The risk of a SPC with a specific exposure as smoking was estimated by comparing the patients without the specific exposure, using Poisson regression methods adjusting for gender, histology (squamous cell carcinoma versus non-squamous cell carcinoma) and cumulative smoking amount before the treatment of NSCLC (40 pack-years > versus $\geqslant$40 pack-years) (15).

RESULTS

The 62 questionnaires completed for each patient showed that none of the patients had past history of cancer of any site nor received previous chemotherapy or RT. The patient characteristics are summarized in Table 1. The end of observation to count the person-years was 31 December 1998. The median follow-up from initiation of therapy was 6.2 years (range 3.1–12.2 years). Of the 62 patients, nine developed SPC in 435 person-years of follow-up. Forty-six patients have remained free of cancer since initial treatment. Three other patients relapsed with NSCLC and still remain alive

Table 1. Patient characteristics ($n = 62$)

Gender	
Male	50
Female	12
Age (median, range)	61, 34–80
Histology	
Squamous cell carcinoma	30
Adenocarcinoma	21
Large cell carcinoma	10
Adenosquamous carcinoma	1
Stage	
IIIA	32
IIIB	30
Surgery	
Yes	24
No	38
Smoking (median, range)	40 pack-years, 0–120
Stop smoking	
Yes	29
No	16
Unknown	17

receiving second line chemotherapy. Of the 62 patients, 13 have died: 5 from recurrent NSCLC, 4 from SPC, 4 from other causes. Regarding chemotherapy for initial treatment, 39 patients were treated with cisplatin (CDDP) + mitomycin (MMC) + vindesine (VDS), 16 with CDDP + VDS, 4 with carboplatin, 2 with CDDP + irinotecan, with 1 with CDDP + MMC + inorelbine. In the treatment of RT, 66 Gy were given to 5 patients, 60 Gy to 10, 56 Gy to 28, 50 Gy to 15 and 40 Gy to 4. Of the 62 patients, surgery was performed in 24 patients after the chemo-radiotherapy.

For smoking status, information was obtained for all the 62 patients before the treatment, but was available for 45 patients after the treatment. Of the 45 patients treated in the analysis, 16 patients continue to smoke and 19 patients stopped smoking. For assessment, 10 never smokers were also added to the 19 stopped patients, and the 29 patients were categorized to the stop smoking group.

Details of nine patients who developed SPC out of the 62 patients are shown in Table 2. There has been no SPC among the ten never smokers. Two patients (cases 5 and 9) developed a SPLC in different lobes from the original NSCLC. Both tumors arose from the ipsilateral side and both patients continued to smoke after the treatment. One of the two lung cancers developed inside the radiation field. The other malignancies consisted of carcinoma of the esophagus, stomach, colon, skin, breast and acute myelogenous leukemia. Two SPC with skin and breast cancer (cases 6 and 8) also developed inside the radiation field.

Table 3 shows the relative and absolute risks of SPC after initiation of therapy for NSCLC. The risk for development of any SPC increased significantly to 2.8 [95% confidence interval (CI) 1.3–5.3]. In spite of the overall increase in risk, there was no significant increase in relative risk of developing a particular cancer. When smoking-related cancers are combined, there was still no significant increased relative risk in the development of SPC.

Table 2. Characteristics of nine patients with second primary cancers

Patient	Age	Gender	CFI (years)	P His	SPT/His	
1	70	M	3.9	LA	Stomach/AD	
2	69	M	11.5	AD	Colon/AD	
3	61	M	6.3	SQ	Esophagus/SQ	
4	65	M	4.5	SQ	Stomach/AD	
5	62	M	5.6	SQ	Lung/SQ	
6	58	M	4.5	AD	Skin/SQ	inside RT field
7	66	M	8.1	SQ	AML	
8	54	F	10.4	LA	Breast/AD	inside RT field
9	66	M	7.9	AD, SQ	Lung/Undiff	inside RT field

CFI, cancer-free interval; P, Primary; His, Histology; AD, adenocarcinoma; LA, large cell carcinoma; SQ, squamous cell carcinoma; Undiff, undifferentiated carcinoma; AML, Acute myeloid leukemia; RT, radiotherapy.

Table 3. Risk of second primary cancers

Site	Obs	E	O/E	95% CI	Absolute risk*
All cancers	9	3.23	2.8	1.3–5.3	238.9
Esophagus	1	0.12	8.6	0.1–47.7	
Stomach	2	0.81	2.5	0.3–8.9	
Colon	1	0.39	2.5	0.1–14.1	
Lung	2	0.50	4.0	0.4–7.2	
Skin	1	0.03	36.2	0.4–201.3	
Breast	1	0.03	36.7	0.4–204.1	
Leukemia	1	0.03	30.9	0.4–171.5	
Smoking-related	5	1.81	2.8	0.9–6.4	

Obs, observed; E, expected.
*Excess risk per 10 000 persons per year.

Table 4. Risk of second primary cancers by histology, gender and smoking status

	Obs	O/E	95% CI	Absolute risk*
Histology				
SQ	4	2.7	0.7–6.9	
Non-SQ	5	2.6	0.9–6.7	
Gender				
Male	8	2.7	1.1–5.3	246.7
Female	1	4.3	0.1–23.9	
Surgery				
Yes	4	3.6	0.9–9.2	
No	5	2.3	0.7–5.4	
Smoking				
≤40 pack-years	2	2.2	0.2–8.0	
≥40 pack-years	7	3.0	1.2–6.2	324.2
Intercurrent smoking				
Yes	3	1.8	0.3–5.9	
No	5	5.2	1.6–11.7	430.5

SQ, squamous cell carcinoma; Obs, observed.
*Excess risk per 10 000 persons per year.

Table 5. Relative risk of second primary cancers estimated by multivariate analysis

Risk factor	Relative risk	95% CI
Cumulative smoking (<40 pack-years/≥40 pack-years)	1.4	0.2–8.4
Intercurrent smoking (yes/no)	2.3	0.5–10.8
Histology (SQ/non-SQ)	3.3	0.2–3.3
Gender (male/female)	1.0	0.1–11.2

SQ, squamous cell carcinoma.

Next, the effect of the passage of time was evaluated. The relative risk for 3–4 years after the treatment was 2.2 (95% CI 0.1–23.9) and 1.8 (95% CI 0.1–23.9) for 5–6 years, and 5.2 (95% CI 1.4–13.2) for at or beyond 7 years. The risk changed with the passage of time and it increased significantly (5.2 times at or beyond 7 years) after the treatment. The absolute risk was 600.1 per 10 000 persons per years.

Table 4 shows the results of univariate analysis on the relative risk for a SPC. The risk was significant but modestly increased relative to the general population in male and more cumulative smoking amount (2.7 times; 95% CI 1.1–5.3 and 3 times; 95% CI 1.2–6.2, respectively). Among those who continued to smoke, there was a significantly increased relative risk (5.2 times; 95% CI 1.6–11.7). In contrast, those who stopped smoking showed only a 1.8-fold increase (95% CI 0.3–5.9), which was not significantly different from the general population.

Finally, we assessed multivariate analysis and examined the relationship between continued smoking habits and the risk of a SPC, adjusted for gender, histology type and cumulative smoking amount. The results are shown in Table 5. We could not demonstrate that factors such as continued smoking habits, gender, histology type and cumulative smoking amount had effect on the development of a SPC.

DISCUSSION

There has been a large body of work that evaluated the risk of SPC in the patients with NSCLC in the treatment of surgery or RT alone (5678). Although the number of survivors in patients with stage III NSCLC has increased by combined modality therapy as chemotherapy and RT, there has been no report to date to evaluate the risk of SPC in these patients. Additionally, Ng and co-workers (16) reported that the relative risk of SPC was 6.1 with the combined chemotherapy and RT and 4.0 with the RT alone, showing a significant difference (*P* = 0.03) in the surviving patients in Hodgkin's disease. Given that, we focused on the NSCLC patients treated with chemo-radiotherapy.