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Postoperative pelvic radiotherapy for cervical cancer patients with positive parametrial invasion

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Abstract

Objective. To evaluate patterns of failure in cervical cancer patients with histopathologic parametrial invasion treated with postoperative pelvic radiation therapy.

Methods. Records of 117 stages IB–IIB cervical cancer patients with parametrial invasion treated with postoperative radiation therapy from 1985 to 2002 were retrospectively reviewed. Patients were divided into two groups based on status of pelvic lymph nodes. Patterns of recurrence and prognosis by status of pelvic lymph nods were statistically analyzed.

Results. Status of pelvic lymph nodes had significant impact on both recurrence and survival. Extrapelvic recurrence was observed in 23 of 66 node-positive patients compared with 6 of 51 node-negative patients ($P = 0.005$). Of 66 patients with a positive pelvic lymph node, 18 developed visceral metastases, whereas only three visceral metastases were noted in the 51 node-negative patients ($P = 0.003$). Five-year overall survival in node-positive and -negative patients was 52% and 89%, respectively ($P = 0.0005$). Corresponding rates for recurrence-free survival were 44% and 83%, respectively ($P = 0.0002$). The correlation between nodal metastasis and prognosis was enhanced when node-positive patients were stratified into two groups based on number of positive nodes ($n = 1$ and $n \geq 2$). Five-year recurrence-free survival rates for patients with negative, one positive, and two or more positive nodes were 83%, 61%, and 31%, respectively ($P = 0.0001$).

Conclusions. Extrapelvic recurrence was uncommon in node-negative patients with parametrial invasion. These findings do not support use of systemic therapy for cervical cancer patients with parametrial invasion if pelvic lymph node metastasis is negative.

Keywords: Cervical cancer; Parametrial invasion; Lymph node; Postoperative radiation therapy

Introduction

Adjuvant therapies for women with International Federation of Gynecology and Obstetrics (FIGO) stage IIB cervical cancer have not been widely examined in Western countries because these patients have mainly been treated initially with radical radiotherapy. On the other hand, in Japan, stage IIB cervical cancer patients have been predominantly treated surgically and receive postoperative adjuvant pelvic irradiation if histopathologic examination confirms parametrial involvement. Eligibility criteria of a recent clinical study for IB–IIA disease (which showed a positive survival effect for adjuvant concurrent cisplatin-based chemotherapy and radiation therapy) included parametrial tumor invasion [1]. Thus, it is now recommended that patients with parametrial invasion should receive postoperative concurrent chemotherapy and pelvic radiation therapy. However, this group of patients consists of those with and without pelvic lymph node metastasis. Status of

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pelvic lymph nodes is considered to be one of the most important prognosticators [2–7]. In addition, it is still unclear whether patients with all stages and extents of disease equally benefit from chemotherapy. Therefore, it is still unknown whether this heterogeneous patient group should be uniformly treated with concurrent chemoradiation. Results of our previous study, which included 45 patients with pathologic T2b (pT2b) disease by TNM classification, showed that lymph node status had an obvious impact on development of distant metastasis [8] and thus did not support use of chemotherapy for patients without lymph node metastasis. In order to examine this issue further, in the current study, we reevaluated patterns of failure in stages IB–IIB cervical cancer patients with histopathologic parametrial invasion treated with postoperative pelvic radiotherapy in a larger patient group. The main objective of the present study was to validate or refute our previous speculation that status of pelvic lymph nodes determines the recurrence pattern in patients with parametrial invasion.

Patients and methods

Between April, 1985, and March, 2002, 327 patients with FIGO IB–IIB carcinoma of the cervix received postoperative pelvic radiotherapy at the Chiba University Hospital and three affiliated institutions. Eligibility criteria for the present study included (1) radiotherapy preceded by a class III radical hysterectomy with bilateral pelvic lymphadenectomy; (2) squamous cell carcinoma; (3) histopathologically confirmed parametrial invasion (pT2b); and (4) dose of pelvic radiotherapy ≥40 Gy. A total of 117 patients with pT2b uterine cervical cancer according to the UICC-TNM classification were entered into this study. Patient characteristics are shown in Table 1. Median age was 53 years (range, 27–79). Preoperative FIGO clinical stage was IB for 17 patients, IIA for 6, and IIB for 94. After surgery, a total of 66 patients (56%) were positive for pelvic lymph node metastasis, 8 of 17 (47%) stage IB patients, 4 of 6 (67%) stage IIA patients, and 54 of 94 (57%) stage IIB patients. Of the 66 node-positive patients, 29 had only one positive node and the remaining 37 had two or more positive nodes. A microscopically positive surgical margin was observed in 32 of 117 (27%) patients. The majority of patients were found to have some degree of lymph-vascular space invasion. The retrospective and multicenter nature of the current study placed some limitations on data available for analysis. In general, preoperative bimanual examination has been performed without any anesthesia in Japan, which limited findings for some patients, especially those for whom the exam was painful or otherwise difficult. In addition, preoperative work-up did not necessarily include magnetic resonance imaging (MRI), especially in the early part of the study period. Furthermore, lesion size was not always accurately cited in the pathology report and variability existed in the direction of sectioning for slide preparation. Thus, it was impossible to determine the greatest tumor diameter objectively. Patients with grossly positive nodes were not included in the present study. However, patients with questionable abnormalities by pelvic CT or those with unknown lymph node status before surgery were eligible.

Table 1
Patient characteristics

No. of patients	117
Age	
Median	53
Range	27–79
Clinical stage	
IB[a]	17
IIA	6
IIB	94
Surgical margin	
Negative	85
Positive	32
Lymph node metastasis	
Negative	51
Positive	66
Dose of irradiation (Gy)	
Median	50
Range	40–56

[a] Includes four patients with IB2 disease diagnosed after 1995.

The radiation therapy techniques have been described previously [8], and they were similar at each institution. In brief, patients were treated with 10–18 MV X-rays from a linear accelerator using anterior and posterior opposing techniques, with the fields encompassing the whole pelvis extending from the lower margin of the obturator foramen to the upper margin of the fifth lumbar vertebra and laterally to at least 1.5 cm outside of the true pelvis. After implementation of a computed tomography (CT) simulator with three-dimensional treatment planning system, patients were predominantly treated with four-field box technique. Anterior and posterior borders of the lateral fields were carefully determined based on preoperative diagnostic imaging studies such as CT and MRI, with an adequate coverage of the pelvic lymph node area and the primary tumor bed. Typically, the anterior margin was placed just anterior to the symphysis pubis and the posterior margin included the anterior aspect of the sacrum. No attempt was made to irradiate paraaortic lymph nodes. A fractional daily dose of 1.8–2.0 Gy at midpoint of the central axis or at beam intersection of central axes to a median total dose of 50 Gy (range, 40–56 Gy) was delivered. Patients with a microscopically positive surgical margin at the lateral parametrium could be treated with a boost field up to a dose of 60 Gy. Brachytherapy was applied for women with microscopic tumor cut-through at the vaginal stump at the discretion of the attending physician. In general, a prescribed dose of 500–1200 cGy at the submucosa 5 mm in one to two fractions was delivered by high-dose-rate machines. No patient received systemic chemotherapy

adjunctively. Only six node-positive patients who were treated after 1998 received weekly or daily cisplatin concurrently with pelvic radiotherapy.

Patients were followed every one to 3 months. Mean follow-up of surviving patients was 72 months (range, 6–237 months). Patients were, in general, examined clinically at 1- to 3-month intervals during the first 2 years, at 1- to 6-month intervals for the next 3 years, and yearly thereafter. The diagnosis of recurrence was predominantly based on clinical and radiological findings, with histological confirmation in cases of supraclavicular lymph node metastasis or central vaginal stump recurrence.

Sites of recurrent were divided into pelvis and extrapelvis. Pelvic recurrence was further divided into central and peripheral pelvic wall. Extrapelvic recurrence included paraaortic node recurrence as well as other sites such as supraclavicular lymph nodes and visceral recurrence. For analysis of recurrence pattern, only the site of the first failure, which emerged more than 1 month earlier than recurrence at any other site, was counted. Impact of clinical factors on development of recurrence was examined by chi-square test. A P value of less than 0.05 was considered statistically significant.

Overall survival (OS) was measured from date of surgery to death from any cause, with surviving patient follow-up data censored at the last contact date. OS and recurrence-free survival (RFS) were estimated using the method of Kaplan and Meier. Time-to-event distributions were compared using the log-rank test with a statistical significance level of 0.05. All estimated P values were two tailed. Late bladder and intestinal complications were graded according to the RTOG/EORTC late radiation morbidity scoring system [9]. Probability of developing leg edema was graded by LENT-SOMA scale [10] and was calculated actuarially. Patients who died of intercurrent disease without experiencing leg edema were censored at the time of death. In calculating leg edema rates, patients who developed pelvic recurrence were excluded.

Results

Patterns of recurrence and impact of clinical factors on recurrence

Thirty-five patients developed recurrent disease in a total of 40 sites. Pelvic recurrence developed in 10 patients, and extrapelvic recurrence developed in 29 patients. Four patients are counted in both categories because they developed recurrent disease in both pelvic and extrapelvic sites simultaneously. None of the clinical factors such as FIGO stage, surgical margin status, and pelvic node status influenced development of pelvic recurrence. Neither FIGO stage nor surgical margin status influenced development of extrapelvic recurrence. In contrast, pelvic node status had significant impact on development of extrapelvic recurrence. Sites of recurrence according to pelvic node status are shown in Table 2. Extrapelvic recurrence was observed in 23 of 66 (35%) node-positive patients as opposed to 6 of 51 (12%) node-negative patients ($P = 0.005$). Notably, 18 of 66 (27%) patients with a positive pelvic lymph node developed visceral metastases, whereas only three visceral metastases were noted in 51 node-negative patients (6%) ($P = 0.003$).

Survival and clinical factors

The 5-year OS and RFS rates for all patients were 69% and 61%, respectively. Surgical margin status did not influence either OS or RFS. Clinical FIGO stage (IB–IIA vs. IIB) had marginal impact on both OS and RFS. Five-year OS rates were 80% and 66% in patients with IB–IIA and IIB disease, respectively ($P = 0.056$). Corresponding figures for RFS were 77% and 58% ($P = 0.067$). Pelvic lymph node metastasis had an obvious impact on both OS and RFS. Five-year OS rates for patients with and without pelvic lymph node metastasis were 52% and 89%, respectively (Fig. 1, $P = 0.0005$). Corresponding figures for RFS were 44% and 83% ($P = 0.0002$). This correlation between status of pelvic lymph nodes and prognosis was enhanced when node-positive patients were classified into two groups based on number of positive nodes ($n = 1$ or $n \geq 2$). Five-year RFS rates for patients with negative, one positive, and two or more positive lymph nodes were 83%, 61%, and 31%, respectively (Fig. 2, $P = 0.0001$). Corresponding figures for OS were 89%, 61%, and 46%, respectively ($P = 0.0011$).

Toxicity

No patient developed a severe life-threatening complication that required surgical intervention during radiotherapy. As for late bladder complications, grade 2 complications were observed in three patients and a grade 3 complication in one patient. Grade 2 intestinal complications requiring conservative treatment occurred in three patients. Grade 3 intestinal complications requiring surgical intervention occurred in two patients. Use of brachytherapy did not influence development of complications. The most

Table 2
Sites of recurrence based on status of pelvic lymph nodes

Node status	Pelvis		Extrapelvis		Total
	Central	Peripheral	PAN	Visceral recurrence	
Negative ($n = 51$)	1	3[a]	3	3	10
Positive ($n = 66$)	2[a]	4[b]	6	18[c]	30
Total	3	7	9	21	40

PAN, paraaortic lymph nodes.
[a] One patient had simultaneous extrapelvic recurrence.
[b] Two patients had simultaneous extrapelvic recurrence.
[c] One patient also had PAN metastasis.

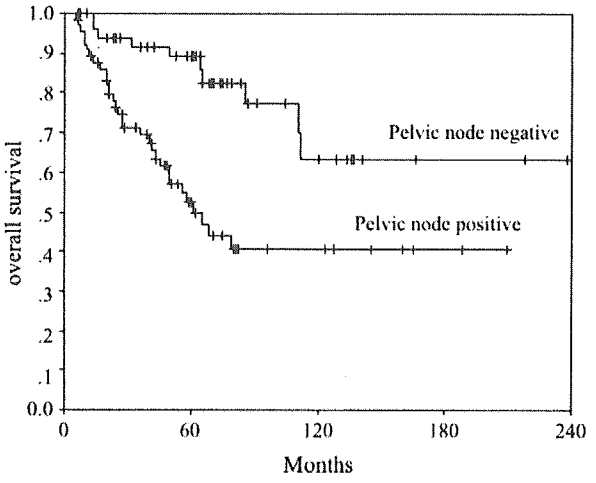

Fig. 1. Kaplan–Meier estimates of overall survival for cervical cancer patients with parametrial invasion based on status of pelvic lymph nodes.

common complication was lymphedema. Because of limitations due to this study's multi-institution retrospective setting, leg edema could be assessed in only 57 patients. Of those, 12 patients developed leg lymphedema with symptomatic edema (grade 2) in four patients and secondary dysfunction (grade 3) in eight. The actuarial probability of suffering leg edema at 3 years was 26%.

Discussion

Women with FIGO stage IIB cervical cancer are considered to have advanced lesions. Even if they can be surgically treated, postoperative histological examination typically reveals various risk factors for recurrence that mandate additional local and/or systemic therapies for the majority of cases. The Milan trial, which compared survival outcome for patients with stages IB–IIA cervical cancer randomized to radiation or radical surgery with selective adjuvant postoperative radiotherapy, showed that the rate of late complications was highest among patients undergoing combined modality therapy with identical survival outcome. Patients staged greater than IIA are not candidates for initial surgery in the United States and Europe because the majority will need postoperative radiotherapy that increases the probability of developing late complications. However, initial surgery for IIB disease is the usual practice in Japan and we believe this practice pattern will continue. Because Japanese women present first at gynecologic clinics, it is gynecologists in most institutions who determine treatment strategy without additional input from radiation oncologists. Most gynecologists consider surgical treatment to be superior to radiotherapy; as a result, the majority of patients with stage IIB cervical cancer have been treated with radical hysterectomy and lymphadenectomy. These patients have also typically received postoperative pelvic irradiation if histopathological examination confirmed pT2b disease or other risk factors.

The concept of postoperative pelvic radiotherapy, therefore, is quite different between the United States/Europe and Japan. In the United States/Europe, postoperative radiotherapy means "adjuvant pelvic radiotherapy for completely resected stages IB–IIA disease" whereas, in Japan, it simply means "radiotherapy after surgery." Thus, most Japanese postoperative studies include patients with initial FIGO stage IIB disease, for whom initial chemoradiation is now the treatment of choice in the United States/Europe. Comparison of the two different approaches, initial chemoradiotherapy versus surgery followed by adjuvant radiotherapy, is beyond the scope of the present study. One benefit of the latter strategy is that surgical lymph node staging might select the subpopulation of patients who can safely skip chemotherapy.

Based on five recent randomized trials, it has become increasingly clear that concurrent chemoradiation is more effective than radiation alone for most clinical stages of cervical cancer [1,11–14]. Concurrent cisplatin-based chemotherapy with radiation therapy has become the standard of care in advanced cervical cancer and a standard for some patients receiving adjuvant therapy after initial surgery. Eligibility criteria of the adjuvant chemoradiation trial included parametrial invasion [1]. Results of that study suggest that patients with parametrial invasion should undergo postoperative concurrent chemotherapy and pelvic radiation therapy. In the present study, extrapelvic recurrence was dominant in patients with positive pelvic lymph nodes. Thus, systemic therapy should be offered for patients with node-positive pT2b disease.

Many investigators have shown that survival after radical hysterectomy is affected by lymph node status [2–7]. Patients with positive pelvic lymph nodes were placed in the high-risk group in the Gynecologic Oncology Group (GOG) study. Despite the finding that extrapelvic recurrences were dominant in the node-positive group, this did not translate into a decreased survival outcome in our

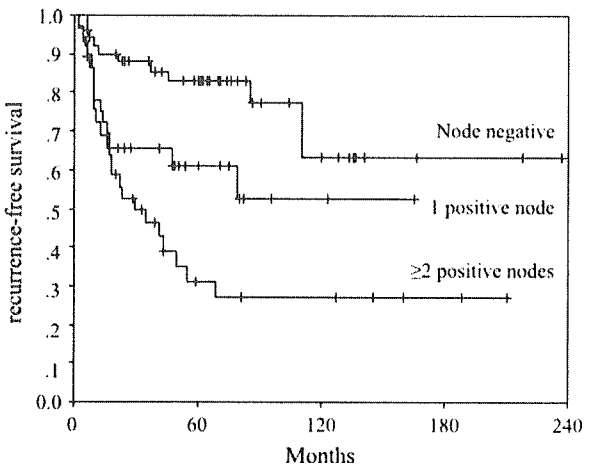

Fig. 2. Kaplan–Meier estimates of recurrence-free survival for patients with negative, one positive, or two or more positive pelvic lymph nodes.

previous study, probably due to the small number of patients [8]. In the present study, which had a larger patient population, pelvic lymph node metastasis had a significant impact on both OS and RFS. There are other primary tumor-related risk factors such as lesion size, depth of stromal invasion, and capillary-lymphatic space invasion. Thomas and Dembo postulated that women with negative nodes but primary tumor-related poor prognostic factors were more likely to develop recurrent disease in the pelvis and therefore might benefit from pelvic irradiation [15]. The randomized study conducted by the GOG demonstrated the efficacy of postoperative pelvic irradiation for patients with IB disease with tumor-related pathologic risk factors other than pelvic lymph node metastasis [16]. Two-year recurrence-free rate in the study was 88% in patients who received pelvic radiotherapy compared with 79% in the surgery alone group ($P = 0.008$). In the present study, only 10 patients developed pelvic recurrence. Although the majority of patients belonged to stage IIB, pelvic control in the present study appears to be identical with findings from other studies that mainly treated patients with stages IB–IIA lesions [1,16–20]. According to the GOG criteria, all node-negative patients in the present study can be allocated to the intermediate-risk group. Therefore, it may be a reasonable extrapolation that adjuvant pelvic irradiation effectively reduces the number of recurrences in women with node-negative pT2b disease. The present results suggest that parametrial invasion per se is not a definitive indication for combined systemic chemotherapy and radiotherapy. Only 6 of 51 node-negative patients developed extrapelvic recurrence as opposed to 23 of 66 node-positive patients. Notably, only 3 of 51 node-negative patients developed visceral metastases. From these data, it appears that parametrial involvement without pelvic lymph node metastasis should not be overestimated as a risk factor for recurrence outside the pelvis. Because the superiority of combined modality therapy in the adjuvant setting was mainly demonstrated in patients with stages IB–IIA disease with lymph node metastasis, there are no relevant data for IIB disease treated with initial radical surgery. Treatment strategies for histopathologically confirmed parametrial invasion in several Eastern countries such as Japan, where patients with IIB cervical cancer are predominantly treated with initial surgery, require further investigation. Results of the present study suggest that adjuvant therapy for pT2b should be tailored according to pathological lymph node status, although this should be confirmed with a prospective study.

In conclusion, the results of the present study strongly support the use of concurrent chemoradiation in node-positive pT2b patients. However, the role of systemic chemotherapy for patients without lymph node metastasis remains questionable. For patients with parametrial involvement without lymph node metastasis, postoperative pelvic radiotherapy can offer both sufficient pelvic control and survival.

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Caffeine Sensitizes Nondividing Human Fibroblasts to X Rays by Inducing a High Frequency of Misrepair

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Kawata, T., Ito, H., Saito, M., Uno, T., Okayasu, R., Liu, C., Kan'o, M., George, K. and Cucinotta, F. A. Caffeine Sensitizes Nondividing Human Fibroblasts to X Rays by Inducing a High Frequency of Misrepair. *Radiat. Res.* 164, 509–513 (2005).

Caffeine sensitizes cells to ionizing radiation, and this effect is believed to be associated with the disruption of DNA damage-responsive cell cycle checkpoints, which is controlled by ATM. Recent studies suggest that misrejoining of DSBs is one of the underlying mechanisms of AT cell hyper-radiosensitivity. In this study, we investigated the effects of caffeine and radiation on nongrowing G_0 normal human fibroblast cells by determining cell survival and scoring aberrations in calyculin A-induced G_2 chromosomes. Results from the cell survival study indicate that after X-ray exposure G_0 cells were sensitized by 24 h treatment with caffeine. Analysis of chromosome aberrations using FISH (fluorescence *in situ* hybridization) revealed a high frequency of aberrant cells and color junctions in the caffeine-treated cells. Since most DNA repair in nongrowing G_0 cells is believed to result from nonhomologous end joining (NHEJ), caffeine may influence the fidelity of the NHEJ pathway in irradiated G_0 cells. © 2005 by Radiation Research Society

INTRODUCTION

Ataxia telangiectasia (AT) is a human autosomally recessive syndrome characterized by cerebellar ataxia, telangiectases, immune dysfunction, genomic instability, and a high cancer incidence (*1, 2*). AT cells are abnormally sensitive to ionizing radiation (*3, 4*), as can be seen from many indicators, including an increased level of chromosome aberrations in cells after exposure (*5–8*). The diverse clinical features in individuals affected by AT and the complex cellular phenotypes are all linked to the functional inactivation of a single gene (*ATM*, AT mutated) (*9*). Several theories have been proposed for the underlying processes involved in this radiation hypersensitivity, including loss of cell cycle arrest (*10–13*), inappropriate TP53-mediated apoptosis (*13*), DNA or chromosomal repair deficiency (*5, 6, 14–21*), impaired accuracy of DNA or chromosome break rejoining (*22–24*), and both repair deficiency and impaired accuracy of break rejoining (*25*).

Caffeine is well known as an efficient inhibitor of two key checkpoint-regulating proteins, ATM and ATR (*26, 27*), and has been shown to sensitize cells to ionizing radiation (*28–33*). Ionizing radiation induces DNA damage, which triggers cell cycle checkpoint activation and consequently cell cycle arrest. Cell cycle arrest is believed to provide an extended time for cells to repair DNA damage before they undergo cell division. Since the checkpoint defects induced by caffeine are reminiscent of the checkpoint defects seen in AT cells, this disruption of checkpoints is assumed to result in the radiosensitizing effects produced by this chemical. Therefore, most previous studies have been performed using exponentially growing cells to study the influence of caffeine on cell cycle checkpoints, with relatively few studies focusing the effects of caffeine on nongrowing G_0 cells. However, Iliakis and coworkers (*34*) have clearly demonstrated the radiosensitizing effects of caffeine on nongrowing cells using a colony formation assay. Natarajan and coworkers (*35*) also reported that chromosome aberrations in G_0 lymphocytes treated with caffeine after irradiation have a tendency to increase, although individual differences in sensitivity were observed. These results indicate that caffeine has biological effects other than those that affect cell cycle checkpoints. If ATM is inhibited by caffeine, an increased induction of misrejoining will be observed in G_0 cells that have been treated with caffeine after irradiation, as was observed in AT cells (*22–25*).

In the present study, we investigated the effect of caffeine on cell survival and chromosome break repair in normal human fibroblast cells in G_0. We assessed the fidelity of chromosome break repair by scoring aberrations in FISH (fluorescence *in situ* hybridization)-painted chromosomes.

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MATERIALS AND METHODS

Cells

Cells of the normal human fibroblast cell lines AG1522 and AG1523 were obtained from the NIA cell repository. Low-passage AG1522 and AG1523 cells were grown in minimum essential medium (MEM) supplemented with 15% fetal bovine serum. Cells were plated into T-25 flasks at 25% confluence and grown for 5 days before being irradiated in the confluent state. The cell density was measured using a Coulter counter at the time of irradiation and after 24 h incubation. There were approximately 1.5×10^6 cells per T-25 flask at the time of irradiation, and no significant increase in this cell density was detected 24 h later, indicating that most cells were not cycling. The percentage of cells in G_0/G_1 phase was measured by cytofluorometry and was 93% for each cell line.

Irradiation and Chemicals

X irradiations were performed with an MBR-1520R-3 (Hitachi, Japan) generator operated under 150 kVp and 20 mA with a 1-mm aluminum filter. The dose rate was about 2 Gy/min. Flasks were kept on ice before irradiation, and all the irradiations were carried out at room temperature. Caffeine (Wako Chemicals, Japan) was dissolved in PBS and added to the cell cultures just after irradiation. The concentration of caffeine was adjusted to 5 m*M* or 10 m*M*.

Colony Formation

Confluent cells were exposed to X rays (0–8 Gy) and then were allowed to repair at 37°C for 24 h with either 5 or 10 m*M* caffeine or without caffeine. After incubation, the medium was removed and the cells were washed twice with PBS to remove the caffeine. Cells were then trypsinized and plated onto 100-mm-diameter plastic dishes containing caffeine-free medium to determine colony formation capacity. The cell suspension was counted using a Coulter counter, and the number of cells seeded was adjusted to yield 100 colonies per 100-mm dish. Surviving cells were determined from the number of colonies containing a minimum of 50 cells.

Chromosome Aberrations

A chemically induced PCC (premature chromosome condensation) technique with calyculin A was used to collect chromosomes in the G_2 and mitotic phases of the cell cycle (*24, 36, 37, 38*). Calyculin A can induce PCC effectively in G_2 phase of the cell cycle. After exposure of confluent cells to 6 Gy of X rays, cells were returned to the incubator for 24 h for repair with or without caffeine. After 24 h, the medium was removed from the flasks and the cells were washed twice with PBS as described above. Cells were then trypsinized and transferred from a T-25 flask to a T-75 flask to allow growth. When nongrowing AG1522 cells were exposed to 6 Gy, the G_2/metaphase index was found to peak at around 36 h after subculture (*24*). We therefore elected this time for collecting the first cell cycle postirradiation G_2/metaphase chromosomes. After incubation for 36 h after subculture, calyculin A (Wako Chemicals; final concentration 50 n*M*) was added and the cells were incubated for 30 min at 37°C to allow chromosome condensation. After treatment with calyculin A, cells were transferred to a tube and centrifuged for 5 min at 2000 rpm. The pellet was carefully resuspended in 8 ml of 75 m*M* KCl and incubated at 37°C. After 20 min, 2 ml of freshly prepared fixative solution (methanol:glacial acetic acid = 3:1 vol/vol) was slowly added to the solution, and the tubes were centrifuged again. A final wash and fixation in fresh fixative was completed before the cells were dropped onto a glass slide. Cells were aged overnight at 37°C on a slide warmer and then hybridized *in situ* with fluorescent DNA whole probes 1 (Green) and 3 (Orange) (Vysis). Cells were counterstained with DAPI, and chromosome aberrations were viewed with a Zeiss Axioskop fluorescence microscope. Aberrations in FISH-painted chromosomes were analyzed in one sample of cells irradiated with each dose.

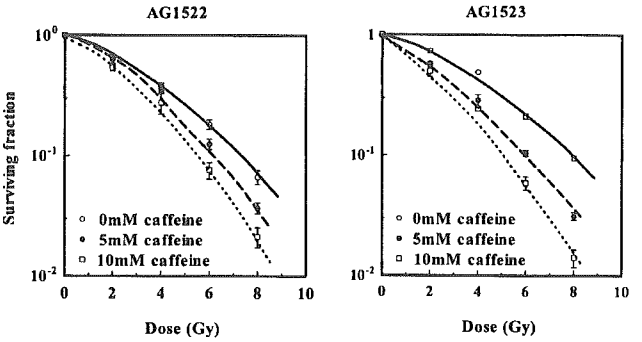

FIG. 1. Survival curves for nongrowing AG1522 and AG1523 cells treated with 0, 5 or 10 m*M* caffeine after irradiation. Each point represents the mean and the standard error (SE) of three independent experiments.

Scoring of Chromosome Aberrations

We measured the number of color junctions per cell as a simple parameter representing the frequency of chromosome misrejoining and the number of excess painted fragments to represent non-rejoined breaks. These excess fragments, which would presumably include both interstitial and terminal-type deletions, were included in one category of deletions. Although terminal deletions are true unrejoining breaks, most interstitial deletions are likely to form rings through rejoining of their broken ends. However, we were forced to include both types of deletions due to the difficulty of discriminating them in FISH-painted chromosomes without the use of telomere probes. Bicolor junctions originate from misrejoining of either a FISH-painted chromosome and a DAPI-stained chromosome or two FISH-painted chromosomes. A reciprocal interchange between a FISH-painted chromosome and DAPI-stained chromosome, i.e. dicentrics and translocations, would therefore contain two bicolor junctions, and an incomplete interchange would contain one bicolor junction. Each painted chromosome was treated independently, and a few exchanges involved exchanges between the painted chromosomes. Therefore, a reciprocal exchange between two FISH-painted chromosomes was measured as four bicolor junctions and an incomplete exchange as two bicolor junctions. The percentage of aberrant cells, which gives a direct measurement of the extent of chromosome damage, was calculated as the ratio of the number of aberrant cells and the total number of cells scored. The total numbers of cells scored were 297 (6 Gy, 0 m*M* caffeine) and 269 (6 Gy, 10 m*M* caffeine) for AG1522 cells and 210 (6 Gy, 0 m*M* caffeine) and 135 (6 Gy, 10 m*M* caffeine) for AG1523 cells.

RESULTS

Similar to what was seen previously (*34*), caffeine had no cytotoxic effects on nonirradiated cells up to concentrations of 10 m*M* (data not shown). Figure 1 shows survival data for AG1522 and AG1523 cells exposed to 0, 5 or 10 m*M* caffeine after irradiation at various doses; a concentration-dependent cell killing effect is demonstrated.

Figure 2 shows an example of aberrations in AG1522 cells exposed to 10 m*M* caffeine after irradiation. The figure clearly shows a color junction originating from chromosome 1 and 3. Figure 3a–c shows the number of color junctions per cell and deletions per cell and the percentage of aberrant cells for AG1522 and AG1523 cells with or without caffeine treatment. In cells treated with 10 m*M* caffeine, the percentage of aberrant cells and the number of color junctions per cell are almost twice as high as yields in cells

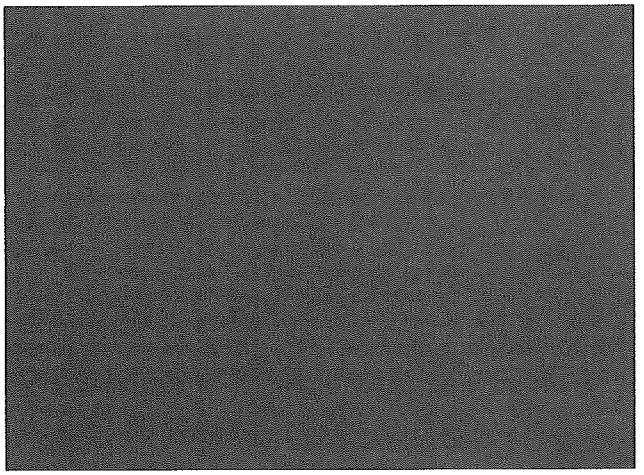

FIG. 2. Example of chromosome aberrations in AG1522 cells after exposure to 6 Gy. Chromosome 1 was painted with a Spectrum Green probe and chromosome 3 with Spectrum Orange probe. Spreads were counterstained with DAPI. Color junction between chromosome 1 and 3 is clearly visible.

without caffeine treatment, while the frequency of deletions per cell was similar for caffeine-treated and nontreated samples. Although chromosome-type aberrations increased in samples treated with caffeine, no significant increase was found in the induction of chromatid-type breaks in either type of cells treated with caffeine after irradiation (data not shown).

DISCUSSION

Our experiments were designed to determine the effects of caffeine on radiation-induced chromosomal damage and repair in nongrowing G_0 human fibroblast cells. Since chromosome aberrations are believed to reflect DSB repair, aberrations in FISH-painted chromosomes indicate the effect of caffeine on DSB repair. To eliminate the effects arising from cell cycle checkpoints, confluent cells were irradiated and allowed to repair for 24 h at 37°C with or without caffeine.

The chromosomal repair process in G_0 can be assessed directly in interphase cells using the conventional fusion PCC technique (*39–42*). Kovacs *et al.* (*42*) irradiated nongrowing AG1522 cells and compared PCC 24 h after irradiation with that in cells that had reached first metaphase after exposure and found that there were fewer in aberrations at metaphase, particularly unrejoined breaks, indicating the importance of scoring aberrations in prematurely condensed chromosomes for assessing the repair at G_0. However, fusion PCC is relatively difficult and the PCC index is low. In the present study, we elected to score aberrations in G_2 chromosomes condensed by calyculin A instead of directly scoring G_0 chromosomes. A recent study comparing the frequency of aberrations in calyculin A-induced G_2 PCC with aberrations in G_0 fusion PCC demonstrated that the frequencies of aberrations were similar (*43*). This indicates that a G_2/M-phase cell cycle delay results in the decreased level of aberrations observed in metaphase and therefore that yields found in the G_2 cells are reflective of the yields of misrepaired DSBs or chromosome breaks formed in G_0.

In mammalian cells, two major repair pathways are known to be involved in the repair of DNA breaks: homologous recombination (HR) and nonhomologous end joining (NHEJ) (*44*). HR, which requires the presence of homologous sequences in a homologous chromosome or in a sister chromatid, is precise (relatively error-free) and is important for the repair of DSBs in late-S and G_2 phase, while the NHEJ pathway is error-prone and mutagenic and is the predominant repair process during G_0, G_1 or early S phase (*45, 46*). In the present study, since nongrowing G_0 cells were irradiated and allowed to repair for 24 h before

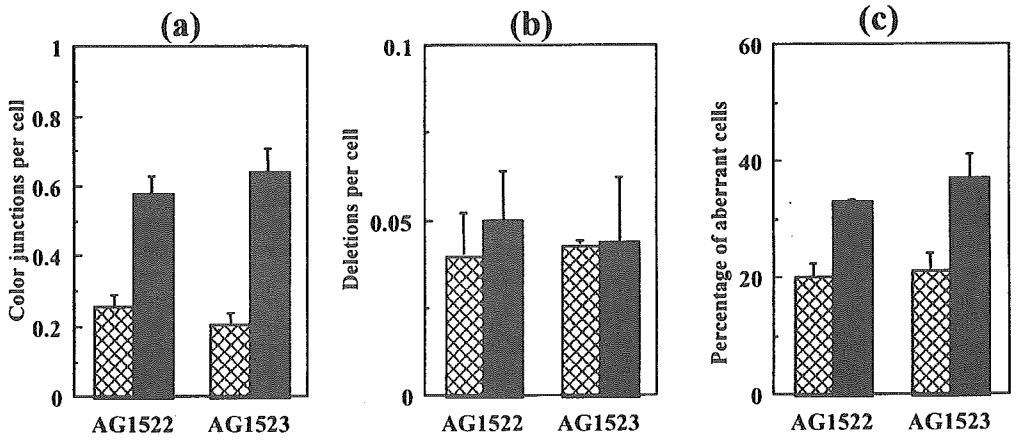

FIG. 3. The effects of caffeine on the induction of color junctions per cell, deletions per cell, and the percentage of aberrant cells. The hatched bar shows the results for samples treated with 0 m*M* caffeine after 6 Gy irradiation. The black bar indicates results for samples treated with 10 m*M* caffeine after 6 Gy. The bars show the standard errors (SE) of the mean.

subculture, it is likely that most DSBs were repaired using the NHEJ repair pathway. The radiosensitizing effect of caffeine on G_0 human fibroblast cells is clearly demonstrated by the colony formation study (Fig. 1). To assess the effect of caffeine on the fidelity of G_0 repair, we scored color junctions and deletions using the FISH technique. Figure 3 shows that in the presence of 10 m*M* caffeine around twice the number of color junctions are induced compared with samples allowed to repair without caffeine, while 10 m*M* caffeine does not affect the frequency of deletions, which include both terminal deletions (unrejoined breaks) and interstitial deletions. These results may indicate that the efficiency of chromosome break rejoining through NHEJ may be independent of caffeine; however, the fidelity of the rejoining process is impaired. These results appear to be different from the previously published finding that caffeine-induced radiosensitization is independent of NHEJ but is mediated through affecting HR (*47–49*). This difference may be attributed to comparison of results obtained under different experimental conditions; i.e., most previous experiments were performed using exponentially growing phase cells, not confluent G_0 cells. In a population of exponentially growing cells exposed to radiation, an accumulation of G_2-phase cells occurs as a result of the G_2 block. Since HR plays an important role in repairing breaks in G_2 phase, the effect of caffeine on the HR pathway could be enhanced and that of NHEJ would be minimized when exponentially growing cells are used. By using synchronized normal human fibroblast cells in G_0, we could clearly demonstrate that caffeine affects the fidelity of repair in G_0 normal human fibroblast cells.

In conclusion, we have shown that the presence of caffeine can result in a high frequency of misrejoining in irradiated G_0 human fibroblast cells. Caffeine may not influence the efficiency of joining breaks through NHEJ, but it can influence the fidelity of repair through NHEJ. Although caffeine is known to be an efficient inhibitor of ATM, caffeine is a relatively non-selective agent and has many effects in cells. For example, caffeine inhibits alkaline phosphatase activity (*50*) and phosphodiesterase activity (*51, 52*). It should therefore be noted that a high induction of misrejoining in G_0 cells after treatment of caffeine would not be attributed solely to the inhibition of ATM. Inclusion of more data from additional cell lines of different radiosensitivity and study using molecular techniques is necessary to confirm these results. Further studies using additional normal and AT heterozygous fibroblast cells are under way to investigate the mechanism of radiosensitization of caffeine in cells exposed to low- or high-LET radiations.

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CASE REPORT

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Weekly cisplatin administration concurrent with radiation therapy for locoregionally advanced nasopharyngeal carcinoma

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Abstract Radiation therapy (RT) with concurrent and adjuvant chemotherapy has been a widely accepted treatment for patients with locoregionally advanced nasopharyngeal carcinoma (NPC). We administered 40 mg/m^2 cisplatin (CDDP) weekly, concurrently with RT, to six consecutive patients with locoregionally advanced NPC to evaluate its toxicity and efficacy. The median number of courses of CDDP administration was 4.5 and the median radiation dose was 69.7 Gy. Grade 3 leukopenia was observed in three patients. All but one patient experienced grade 3 or 4 skin reactions, pharyngitis, or dysphagia. All but one patient achieved a complete response, and the remaining patient received radical neck dissection for persistent cervical lymphadenopathies, which contained no cancer cells. All six patients were disease-free at last contact, with a median follow up of 23.5 months. This regimen is well tolerated in patients with locoregionally advanced NPC.

Key words NPC · Concurrent chemoradiotherapy · Weekly CDDP · Intergroup Study 0099

Introduction

Concurrent chemoradiotherapy is the mainstay of treatment for various malignancies.[1–4] Platinum agents, including cisplatin (CDDP), with or without other agents, are used commonly in this setting; however, an optimal CDDP administration schedule remains to be determined. In patients with nasopharyngeal cancer (NPC), the Intergroup Study 0099 (IGS) has demonstrated significant results by administering 100 mg/m^2 CDDP concurrent with radiation therapy (RT) at 3-week intervals.[5,6] After the publication of the IGS, we attempted to adopt an identical combined modality treatment for patients with locoregionally advanced NPC, between March 2001 and June 2002. Although we treated only three patients according to the this regimen, we failed to demonstrate its feasibility and efficacy, because of its severe acute adverse events, poor compliance, and unsatisfactory outcome.[7]

In contrast to the IGS, a Hong Kong group conducted a phase III randomized trial comparing radical RT with concurrent weekly CDDP and RT.[8] They demonstrated significant improvement of progression-free survival in patients with advanced stages, with 40 mg/m^2 weekly CDDP administration. We report the feasibility and efficacy of this weekly chemotherapy CDDP schedule, given concurrently with RT.

Case report

From July 2002, we have treated six consecutive patients with biopsy-proven stage IIB to IVB NPC. All six patients met the inclusion criteria of Chan et al.,[8] and underwent a complete history, physical examination, complete blood counts, screening blood tests of hepatic and renal function, and 3 consecutive days of 24-h creatinine clearance. The disease evaluation included a chest radiograph; bone scintigraphy; computed tomography (CT) of the head and neck, chest, and abdomen; magnetic resonance imaging (MRI) of the nasopharynx and base of skull; and fiberoptic endoscopy and biopsy of the nasopharynx. The patients were staged according to the 1997 International Union Against Cancer (UICC)-TNM staging system. The patients' characteristics are shown in Table 1. Informed consent was provided according to the Declaration of Helsinki.

The patients received 40 mg/m^2 CDDP weekly during RT, starting on the first day of RT. All patients received adequate hydration and a serotonin antagonist against emesis during the CDDP administration. Chemotherapy was

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delayed until bone marrow suppression recovered, and was suspended if serum creatinine was greater than 1.5 mg/dl, and/or creatinine clearance fell to less than 50 ml/min. No patients were scheduled to receive adjuvant and/or neoadjuvant chemotherapy.

With regard to RT, CT-based treatment planning was used to assess the extent of the primary tumor and the neck nodes. The nasopharynx and the upper neck were treated with two opposed lateral fields. A separate anterior supraclavicular field was used to irradiate the lower neck and supraclavicular fossa. The patients were treated with a combination of 4- and 10-MV photons to achieve dose homogeneity. An electron field of appropriate energy was also applied to treat posterior neck nodes after sparing the spinal cord. The fractional daily dose was 2 Gy, with a planned total dose of 66 Gy.

The response assessment included physical examination, fiberoptic endoscopy, and CT and/or MRI of the nasopharynx and neck, and responses were classified according to the *New guidelines to evaluate the response to treatment in solid tumors*.[9] Acute toxicities were graded according to the National Cancer Institute common toxicity criteria.

All six patients received RT without treatment breaks, with a median dose of 69.7 Gy (range, 64 to 70 Gy). Chemotherapy was delivered in three to six weekly courses, with a median course number of 4.5. The reasons for suspension of CDDP administration included renal toxicity in two patients, and grade 3 leukopenia, pharyngitis, and patient refusal in 1 patient each. The acute toxicity profiles are shown in Table 2. Although three patients developed grade 3 leukopenia, the other hematological toxicities were well tolerated. However, all but one patient experienced grade 3 or 4 skin reactions, pharyngitis, or dysphagia. Body weight loss ranged from 4% to 20.7%, with a median of 14.5%. At the end of the treatment, five patients achieved a complete response, and the remaining patient obtained a partial response; this patient received radical neck dissection for persistent lymphadenopathies; however, histopathological examination revealed no cancer cells in the surgical specimens. All six patients were alive without disease at last contact, with a median follow up of 23.5 months (range, 13 to 27 months).

Table 1. Patient characteristics

	Age (years)	Sex	TNM	Histology
Case 1	49	Male	T4N2M0	WHO III
Case 2	20	Male	T1N3bM0	WHO III
Case 3	59	Male	T4N0M0	WHO III
Case 4	62	Female	T3N2M0	WHO II
Case 5	65	Female	T3N1M0	WHO III
Case 6	74	Male	T2bN1M0	WHO II

Discussion

Concurrent chemoradiotherapy with adjuvant chemotherapy has become standard practice following the publication of excellent results by the IGS.[5,6] Subsequently, several phase II or III studies have demonstrated encouraging results with regard to concurrent chemoradiotherapy with or without adjuvant or neoadjuvant chemotherapy.[8,10–15] In the IGS, patients in the experimental arm received 100 mg/m^2 CDDP as a single agent at 3-week intervals, concurrently with radical RT. In four other studies, patients were also administered 100 mg/m^2 CDDP as a single agent, at 3- or 5-week intervals, concurrently with RT;[10–13] in two of these studies, the CDDP dose was divided equally and given on 4 or 5 consecutive days.[12,13] In our previous study, three patients were to receive the IGS regimen; however, they were not able to complete their planned chemotherapy because of its severe acute adverse events.[7]

In contrast, Chan et al.[8] examined the efficacy of weekly administration of 40 mg/m^2 CDDP concurrently with RT (66 Gy/6.5 week), compared with RT alone, in a randomized phase III trial. They demonstrated that progression-free survival was significantly prolonged in patients with advanced stage disease; however, in the overall comparison, progression-free survival was not different among the treatment arms. This CDDP administration schedule has been shown to have acceptable toxicities, with an encouraging outcome for cervical cancer,[7] and, in light of our previous experiences employing the IGS protocol, we incorporated weekly CDDP administration into the present study. Although, in comparison to our previous study, hematological toxicities were more frequent in the present one, nonhematological toxicities were comparable to those in the previous study,[7] and we obtained encouraging results in the present study.

Table 2. Acute adverse events: maximum grade observed for each patient

	Case 1 Grade	Case 2 Grade	Case 3 Grade	Case 4 Grade	Case 5 Grade	Case 6 Grade
Leukopenia	2	3	1	3	3	1
Anemia	1	1	1	2	3	1
Thrombocytopenia	1	1	1	1	0	0
Weight loss	2 (−13.5%)	2 (−18.8%)	2 (−10.7%)	2 (−15.6%)	0 (−4%)	3 (−20.7%)
Dermatitis	2	2	3	3	2	2
Dysphagia	3	3	2	2	2	3
Pharyngitis	3	4	2	3	2	3

Another concurrent chemoradiotherapy schedule includes daily administration of chemotherapy. This schedule has not been tested in head and neck cancers, including NPC; however, Schaake-Koning et al.[16] demonstrated that 6 mg/m^2 CDDP given daily, in combination with RT, in patients with inoperable non-small-cell lung cancer, improved the survival and local control rates compared with 30 mg/m^2 weekly administration. It is still to be determined whether the same situation would apply in patients with NPC or other head and neck cancers.

Little is known about whether combination chemotherapy is superior to CDDP monochemotherapy in the setting of concurrent chemoradiotherapy. Standard chemotherapy for head and neck cancers is a combination of CDDP and 5-fluorouracil (5-FU). Patients with stage II to IV NPC received concurrent chemoradiotherapy consisting of CDDP plus 5-FU in a Taiwan group study, and it was concluded that concurrent chemoradiotherapy with adjuvant chemotherapy would be the best standard strategy for intermediate-risk patients.[14] Recently, Lin et al.[15] have also demonstrated, in a phase III trial, that concurrent chemoradiotherapy, consisting of 80 mg/m^2 CDDP and 1600 mg/m^2 5-FU as a 96-h continuous infusion, given at 4-week intervals, significantly improved both overall survival and progression-free survival, with acceptable toxicities. However, in patients with cervical cancer, a Gynecologic Oncology Group study failed to demonstrate a survival benefit for a CDDP plus 5-FU regimen compared with weekly CDDP administration, and the combined regimen showed more hematological and gastrointestinal toxicities.[17] Whether the addition of 5-FU to CDDP is more effective than CDDP monochemotherapy for NPC remains to be elucidated. The optimal chemotherapy regimen and appropriate administration schedule, regarding concurrent RT and chemotherapy for the management of locoregionally advanced NPC, remain to be established in future clinical studies. Furthermore, the efficacy and toxicities of new active agents against NPC, such as docetaxel and gemcitabine, remain to be evaluated.[18,19]

In summary, although both our previous[7] and present studies were too small to draw any conclusions, we have suggested that, for CDDP monochemotherapy in a concurrent RT setting, weekly 40 mg/m^2 administration is superior to 100 mg/m^2 delivery at 3-week intervals for Japanese patients with locoregionally advanced NPC, with respect to both efficacy and toxicity profiles. We will extend our experience employing a weekly CDDP administration schedule for the management of locoregionally advanced NPC. We will also incorporate adjuvant chemotherapy to eliminate microscopic metastatic disease in a future prospective trial.

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ORIGINAL ARTICLE

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Phase I study of cisplatin and irinotecan combined with concurrent hyperfractionated accelerated thoracic radiotherapy for locally advanced non-small cell lung carcinoma

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Abstract

Background. Irinotecan, when combined with cisplatin, is an effective treatment for advanced non-small cell lung cancer (NSCLC). This constitutes a rationale for conducting a phase I study of chemoradiotherapy including this combination for locally advanced NSCLC.

Patients and methods. Patients with locally advanced NSCLC and a performance status of 0 or 1 were eligible. The protocol consisted of escalating doses of irinotecan on days 1 and 15, and daily low-dose cisplatin (6 mg/m^2 daily for a total dose of 120 mg/m^2) combined with concurrent hyperfractionated accelerated thoracic irradiation (1.5 Gy twice daily for a total dose of 60 Gy).

Results. The maximum tolerable dose was 50 mg/m^2 of irinotecan, and the dose-limiting toxicity was esophagitis. Tumor response was observed in 50% of cases, and the median survival time of the 12 patients enrolled was 10.1 months, including two patients with 5-year disease-free survival. A pharmacokinetics study demonstrated an accumulation of total platinum, but not of free platinum, during the 26-day treatment period.

Conclusion. The recommended dose for phase II studies was determined.

Key words Locally advanced non-small cell lung cancer · Cisplatin · Irinotecan · Radiotherapy · Phase I study

Introduction

The current standard treatment for locally advanced non-small cell lung carcinoma (LA-NSCLC) consists of platinum-based chemotherapy combined with thoracic radiotherapy.[1] Several randomized controlled trials have shown superiority of the combined modality over radiotherapy alone.[2–7] Some of these studies[2,3,6] eventually reported the clinical relevance of concurrent chemoradiotherapy, and a recent randomized controlled study demonstrated the advantage of concurrent over sequential chemoradiotherapy.[8] A standard protocol defining the most suitable chemotherapeutic agents and radiotherapy schedule, however, has not been established. To improve the efficacy of the combined modality, some researchers have investigated the relevance of multidrug chemotherapy with new agents[9] or hyperfractionated accelerated radiotherapy (HART).[6,10]

HART, in theory, might result in more efficient killing of cancer cells and less damage to normal cells by taking advantage of the differences in repair capacity between them.[11] The advantage of HART over conventional thoracic irradiation has been demonstrated in treating patients with limited-disease small cell lung cancer.[12] Although a recently published study demonstrated a positive statistical trend suggesting a survival advantage with the HART regimen over standard thoracic irradiation, when delivered after two cycles of induction chemotherapy,[13] a clear advantage has never been established in the treatment of patients with LA-NSCLC.

On the other hand, irinotecan (CPT-11) is one of the promising cytotoxic agents for advanced NSCLC. The agent is most active when it is metabolized and converted to the potent topoisomerase I poison SN-38. Its clinical relevance for advanced NSCLC has been suggested by phase II studies.[14–20] A recent phase III study comparing combinations of CPT-11 plus cisplatin and vindesin plus cisplatin, the latter a standard chemotherapy for advanced NSCLC in Japan, has established the clinical relevance of CPT-11.[21] In addition, preclinical studies have demonstrated the synergistic effects of either CPT-11[22,23] or cisplatin[24–27] on irradiation in NSCLC. Interestingly, these synergisms do not necessarily depend on the drug sensitivity of the cancer cells.[22,28] Furthermore, CPT-11 and cisplatin have also been shown to be synergistic.[29,30]

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Therefore, a combination protocol consisting of cisplatin, CPT-11, and concurrent thoracic irradiation could in theory, be expected to be an efficient treatment for LA-NSCLC. Among the combination protocols for LA-NSCLC, Schaake-Koning et al.[2] employed a unique therapeutic regimen consisting of daily cisplatin combined with daily conventional thoracic irradiation that might maximize the potential radiosensitizing effect of cisplatin. They demonstrated a survival advantage in patients treated by low-dose daily cisplatin (6 mg/m^2 per day) over patients treated by weekly cisplatin (30 mg/m^2 per week) when combined with standard thoracic irradiation. The pharmacokinetics of this chronic administration of cisplatin, however, have not been fully investigated. Therefore, we conducted a phase I study based on this protocol, along with a pharmacokinetics analysis, to elucidate the feasibility of a new regimen consisting of daily cisplatin and biweekly CPT-11 combined with HART for patients with LA-NSCLC. As to the dose for HART, Choi et al.[31] determined a maximum tolerated dose (MTD) of 45 Gy in 30 fractions for small cell lung cancer, when combined with the standard dose of chemotherapy consisting of one cycle of cisplatin (33 mg/m^2, days 1–3), cyclophosphamide (500 mg/m^2, day 1), and etoposide (80 mg/m^2, days 1–3), followed by two cycles of cisplatin and etoposide. They also noted, however, that the total dose seemed as important as the dose-intensity in radiotherapy, and that a total dose of 60 to 66 Gy would be needed for a high probability of local tumor control. In fact, HART with a higher dose, 67.6 Gy in 52 fractions in combination with low-dose daily chemotherapy consisting of carboplatin and paclitaxel, is reportedly safe and effective for LA-NSCLC.[32] Therefore, the present study employed a fixed dose (60 Gy, twice daily, in 40 fractions) for HART and a fixed dose of cisplatin (6 mg/m^2, daily), based on the Schaake-Koning's protocol, with an escalating dose of irinotecan.

Patients and methods

Patient eligibility

Patients meeting the following inclusion criteria were enrolled in the study: (1) histologically or cytologically proven NSCLC; (2) unresectable stage III disease; (3) age 15 to 75 years; (4) Eastern Cooperative Oncology Group (ECOG) performance status 0 or 1; (5) no prior chemotherapy or thoracic radiotherapy; (6) measurable lesions; (7) adequate bone marrow function (leukocyte count ≤12,000/μl and ≥4000/μl; hemoglobin ≥10.0 g/dl, platelet count ≥100,000/μl), renal function (creatinine ≤1.5 mg/dl; creatinine clearance ≥50 ml/min), hepatic function (bilirubin ≤1.5 mg/dl, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ≤ twice the upper limit of normal), and pulmonary function (PaO_2 ≥70 torr; no interstitial pneumonia demonstrated on chest roentgenogram); and (8) written informed consent. Exclusion criteria were patients with (1) extended lesions not containable in an irradiation field as determined below; (2) malignant pleuritis, pericarditis, or ascites; (3) previous or concomitant malignancy; (4) any serious complication (such as infectious disease, pseudomembranous colitis, diarrhea, ileus, uncontrolled angina pectoris, acute myocardial infarction less than 3 months previously, cardiac insufficiency, or uncontrolled diabetes mellitus); (5) past history of severe allergic reaction to any medication; (7) pregnancy or breast feeding; or (8) any other disqualifying conditions. The study fully complied with local regulations.

Chemotherapy and evaluation of toxicity and tumor response

Chemotherapy consisted of cisplatin (fixed dose of 6 mg/m^2 per day) × (5 days/week) × 4 weeks to reach 120 mg/m^2 in total, and CPT-11 (escalating dose) on days 1 and 15. CPT-11 was dissolved in 500 ml of saline and delivered intravenously in 90 min. Cisplatin was diluted in 100 ml of saline and delivered intravenously in 30 min. Cisplatin administration was started 60 min after the start of irinotecan administration to complete both agents simultaneously on days 1 and 15. Oral ondansetron at 4 mg or granisetron at 2 mg was given as prophylaxis for nausea/vomiting with every cisplatin administration. Daily chemotherapy was completed approximately 30 min before thoracic irradiation. The first three patients were entered into the first level, from which CPT-11 administration was excluded. In the second level, the CPT-11 dose was set at 40 mg/m^2, with escalations set at increments of 10 mg/m^2. Dosage was escalated in successive cohorts of three new patients as long as the dose-limiting toxicity (DLT) was not encountered in the three patients enrolled in the same level. If DLT was observed in two or more patients in the cohort, this dose level was defined as the MTD. If DLT was found in one patient out of the three, three additional new patients would be treated at the same dose level, and the dose level would be escalated to the next level if none of these three patients experienced DLT; otherwise the dose level would be defined as MTD. DLT was defined as grade 3 or 4 nonhematological toxicity excluding nausea/vomiting and alopecia, or grade 4 hematological toxicity according to the National Cancer Institute Common Toxicity Criteria version 2.0. Tumor response was evaluated according to the Response Evaluation Criteria in Solid Tumors of the National Cancer Institute.

Radiotherapy

For all patients, radiotherapy was delivered using a linear accelerator with a 10-MV photon beam. For the first two patients, an X-ray simulator was used for the treatment planning. For the rest of the patients, after the introduction of the computed tomography (CT)-simulation system, CT-based three-dimensional treatment planning was performed. No tissue heterogeneity correction, however, was used to calculate a prescribed dose, uniformly throughout the study. Radiotherapy consisted of twice daily thoracic irradiation (1.5 Gy at the midplane, two times/day) × (5 days/

week) × 4 weeks to reach 60 Gy in total). Although the original protocol required a minimum interfraction interval of 4 h each day, an interval of at least 6 h was obtained for all, eventually, because of our institutional standard operating procedures for radiotherapy. The original irradiation volume included all of the involved lesions, the ipsilateral hilum, the superior mediastinum, and the subcarinal region, with a margin of 2 cm in a single field. If supraclavicular lymph nodes were involved, only the involved side was included. The irradiation field was reduced to spare the spinal cord when the accumulated radiation dose reached 39 Gy.

Pharmacokinetics study of CPT-11 and cisplatin

In patients who gave additional informed consent for the pharmacokinetics studies of cisplatin and CPT-11, venous blood samples were collected in heparinized tubes at the following time points of days 1 and 15: before the start of administration of the drugs and at 0.5, 1.5, 2.0, 2.5, 3.5, 5.5, 9.5, 13.5, and 24 h after the completion of their administration. For cisplatin, additional sets of plasma samplings, before and 30 min after the start of cisplatin administration, twice a week, were performed. All samples were immediately centrifuged at 3000 rpm for 20 min to isolate the plasma. For cisplatin, a 2-ml portion of each plasma gradient was then placed on a Centrifree MPS-3 conical filter (Amicon, Lexington, MA, USA) and centrifuged again at 3000 rpm for 20 min to eliminate existing protein and protein-bound platinum. Filtered and unfiltered samples were stored at −70°C until measurement. These samples were measured for platinum concentrations by flameless atomic absorption spectroscopy using the same instrumentation and method as reported earlier.[33] By this analysis, the lowest detectable total and free platinum concentration was 50 and 25 ng/ml, respectively. For CPT-11 and its derivatives, the plasma samples were measured for CPT-11, SN-38, and SN-38-glucuronide (SN-38G) by means of high-performance liquid chromatography, and the lowest detection limits were 54, 2 and 2 ng/ml, respectively. The measured concentrations of the derivatives were fitted to a noncompartmental model. All pharmacokinetics parameters calculated on days 1 and 15 were compared by Student's paired t test, and the differences were judged as statistically significant when the P value was 0.05 or less.

Results

Patients enrolled and determination of MTD

Initially, a total of 12 patients were enrolled in this study between April 1995 and July 1999. Among the 12, one patient, of level 2, with the primary tumor adjacent to thoracic vertebrae was judged, in the course of the treatment, to be ineligible because the irradiation field could not be set so as to spare the spinal cord upon reaching a total dose of 39 Gy. Another patient, of level 3, at day 18, refused to continue the study because of grade 2 esophagitis. Therefore, only the remaining ten patients were analyzed for dose escalation, whereas all 12 patients were analyzed for toxicity, tumor response, and intent-to-treat survival. Level 1 was accomplished without DLT by three patients, and level 2 was completed without DLT by four patients, including the one ineligible patient. As the second patient in level 3, however, presented grade 3 esophagitis, one additional patient was treated at this dose level. However, because this fourth patient refused to continue the treatment, as mentioned above, one additional patient was treated at this dose level. As a consequence, two out of the four patients who completed level 3 experienced grade 3 esophagitis, that is, DLT. Therefore this dose level was defined as MTD, and the preceding level (40 mg/m^2 CPT-11 on days 1 and 15, combined with daily cisplatin and twice daily radiotherapy) was accepted as the recommended dose level. The characteristics of the 12 patients according to dose level are summarized in Table 1.

Dose intensity

Among the ten patients analyzed for dose escalation, all patients of levels 1 and 2 were completely treated without any delay. The second patient of level 3, however, discontinued the treatment on day 23 (100% CPT-11, 80% cisplatin, and 80% radiotherapy of the scheduled doses) because of grade 3 esophagitis (DLT). The third patient of level 3 had a 14-day treatment delay because of grade 2 thrombocytopenia, but then completed the entire protocol. The fifth patient of level 3 experienced grade 3 esophagitis, that is, DLT, just at the end of the full-dose protocol.

Pharmacokinetics study of CPT-11 and cisplatin

Six patients, one in level 2 and five in level 3, gave additional informed consent for their entry into the pharmacokinetics study for cisplatin and CPT-11. One other patient in level 2 also consented, but only for CPT-11. The pharmacokinetics parameters of CPT-11 and its derivatives at days 1 and 15 are summarized in Table 2. There was no statistically significant difference between the parameters on days 1 and 15. As to cisplatin, some important pharmacokinetics parameters, including the area under the curve, were not calculated because it was repeatedly administered with the previous trough value still significantly high. Therefore, time–concentration curves of total and free platinum were drawn (Fig. 1). Total platinum concentration significantly increased, finally reaching a maximum concentration of more than 1 μg/ml. In contrast, free platinum decreased in concentration to less than the minimum detection level (25 ng/ml) at 24 h after every repeated administration, and no concentration-related accumulation trend was found (Fig. 1).

Tumor response and survival

A tumor response was observed in five of the ten patients analyzed for dose escalation, and in six out of the total of 12

Table 1. Patient characteristics and summary of treatment results

Case no.	Dose level	Sex	Age	PS	Histology	Clinical stage	Response	Toxicities[b]						First site of relapse
								Neut	Hb	Plt	Eso	Diarr	N/V	
1	1	M	63	1	Ad	IIIB	SD	1	1	0	1	0	1	Bone
2	1	M	70	0	Ad	IIIA	PR	3	1	1	1	0	0	Primary
3	1	M	61	1	Ad	IIIB	PR	2	2	1	1	0	0	Primary
4	2	M	60	0	Ad	IIIB	PD	2	1	2	1	0	0	Lung
5	2	M	72	0	Sq	IIIA	SD	2	2	1	1	0	2	No relapse
6[a]	2	M	63	1	Sq	IIIB	SD	1	1	0	0	0	1	Bone
7	2	M	47	1	Ad	IIIA	PR	0	1	0	1	1	3	Salivary gland
8	3	M	66	1	Sq	IIIB	PR	2	2	1	2	0	0	Primary
9	3	F	59	0	Ad	IIIB	PR	2	2	0	3[c]	0	1	Brain
10	3	F	63	0	Ad	IIIB	SD	2	2	3	2	0	0	No relapse
11[a]	3	M	63	0	Ad	IIIB	PR	1	1	0	2	0	1	Lung and brain
12	3	M	66	0	Ad	IIIB	SD	2	1	0	3[c]	0	0	Primary

Neut, neutropenia; Hb, hypohemoglobinemia; Plt, thrombocytopenia; Eso, esophagitis; Diarr, diarrhea; N/V, nausea/vomiting; PS, performance status; SD, stable disease; PR, partial response; PD, progressive disease; Ad, adenocarcinoma; Sq, squamous cell carcinoma
[a] Ineligible because of unfit irradiation field (case 6) or patient's refusal to continue the protocol (case 11)
[b] Graded by NCI-CTC, version 2.0
[c] Dose-limiting toxicity

Table 2. Comparison of pharmacokinetics parameters of irinotecan derivatives between days 1 and 15

		T1/2 (h)	Cmax (ng/ml)	AUC (ngh/ml)	CL (l/h per meter^2)	Vdss (l/m^2)
Level 2	(n = 2)					
CPT-11	Day 1	8.0 ± 4.3	324.0 ± 42.4	1221.3 ± 180.3	18.9 ± 10.2	184.3 ± 12.2
	Day 15	4.8 ± 0.3	694.0 ± 377.6	1892.5 ± 18.7	17.2 ± 0.1	101.4 ± 27.9
SN-38	Day 1	13.2 ± 0.7	10.1 ± 2.9	99.7 ± 33.3	NA	NA
	Day 15	52.6 ± 60.1	11.1 ± 3.5	85.5 ± 23.1	NA	NA
SN-38G	Day 1	12.9 ± 4.2	37.1 ± 7.3	374.6 ± 190.6	NA	NA
	Day 15	16.8 ± 9.9	42.4 ± 13.1	367.0 ± 146.6	NA	NA
Level 3	(n = 5)					
CPT-11	Day 1	5.5 ± 0.8	383.2 ± 41.0	1736.7 ± 368.1	22.9 ± 4.3	145.8 ± 15.4
	Day 15	5.7 ± 2.8	427.2 ± 69.4	2067.0 ± 803.5	21.5 ± 6.9	143.6 ± 20.4
SN-38	Day 1	14.2 ± 3.6	16.9 ± 5.7	125.8 ± 28.9	NA	NA
	Day 15	9.0 ± 4.9	16.1 ± 3.4	147.9 ± 28.8	NA	NA
SN-38G	Day 1	16.0 ± 11.5	27.1 ± 8.9	222.3 ± 59.8	NA	NA
	Day 15	10.1 ± 7.3	26.0 ± 4.0	257.4 ± 62.0	NA	NA

Mean and standard deviation of the pharmacokinetics parameters are presented in each column. There is no statistically significant difference between days 1 and 15 in any of the parameters (Student's paired t test)
T1/2, half-time; Cmax, maximum concentration; AUC, area under the curve from 0 to 25.5 h of administration; CL, total clearance; Vdss, volume of distribution

patients (Table 1). The first site of relapse in the 12 patients was the primary site in four patients and distant site in six patients. In the other two patients, no relapse occurred (Table 1). As to intent-to-treat survival, median survival time (MST) was 10.1 months, with 1-year and 2-year survival rates of 50% and 25%, respectively. Two patients, cases 5 and 10, survived for more than 5 years without any evident disease progression (Fig. 2).

Late toxicity

Nine patients encountered late toxicities. Briefly, evident pulmonary fibrosis accompanied by partial atelectasis was observed in 8 out of 11 and in 3 out of 4 still living patients at 6 and 12 months from the start of treatment, respectively. Benign pleural effusion was observed in 3 out of 11 and in 1 out of 4 patients at 6 and 12 months from the start of treatment, respectively. No symptomatic esophageal stenosis, benign pericarditis, or cardiac failure was observed.

Discussion

The MTD of CPT-11 administered on days 1 and 15, in combination with daily cisplatin of 6 mg/m^2 for 4 weeks (5 days/week, 20 administrations resulting in 120 mg/m^2 in total) and HART of 60 Gy (in 40 fractions, twice/day) during the same period as cisplatin, was determined in this study. Schaake-Koning et al.[2] reported that daily cisplatin with concurrent conventional thoracic radiotherapy for patients with LA-NSCLC gave a survival advantage over thoracic radiotherapy alone. The present protocol was based on theirs, with the addition of CPT-11. However, in contrast

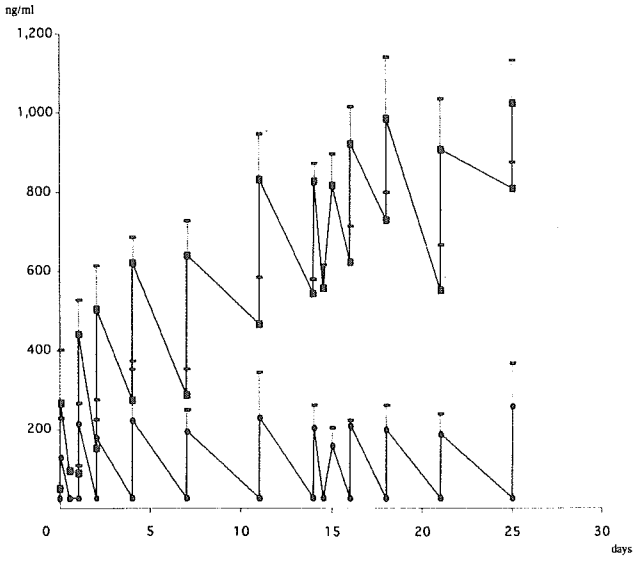

Fig. 1. Mean concentrations of free and total platinum with daily (5 days a week) administration of 6 mg/m^2 of cisplatin for 4 weeks ($n = 6$). The *dotted vertical lines* beyond the means represent standard deviations. Free platinum (*circles*) reached its maximum concentration with every administration and then dropped to a level under the minimum detection limit each time. In contrast, the concentration of total platinum (*squares*) accumulated with repeated administrations, resulting in as much as 1020 ± 109 ng/ml at day 26 and an unusually high rate of protein-bound platinum

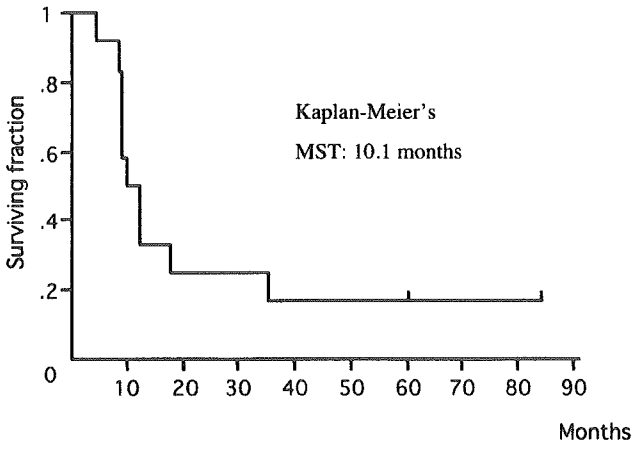

Fig. 2. The intent-to-treat survival curve (Kaplan-Meier's method) revealed 10.1 months of median survival time (*MST*) with two patients being 5-year progression-free survivors out of the 12 patients enrolled

to their protocol, which included a 3-week-interval, the present one did not contain a split radiotherapy schedule during the treatment period. Thus, we conducted a dose escalation test of the protocol, initially by omitting CPT-11 administration (level 1). As this level proved to be feasible, 40 mg/m^2 of CPT-11 was administered on days 1 and 15 (level 2). This dosage was a reduction of the conventional dose,[14,16] 60 mg/m^2 on days 1, 8, and 15, when combined with full-dose cisplatin on day 1 without concurrent thoracic radiotherapy. Thereafter, the CPT-11 dose was planned to be elevated at 10 mg/m^2 increments. As a consequence, level 3 was defined as MTD, because DLT was observed in two of the four eligible patients. The DLT consisted of grade 3 esophagitis in both of these patients. Bone marrow suppression and other toxicities, however, were not severe in any of the patients.

The recommended dose level, level 2, consisted of 40 mg/m^2 of CPT-11 on days 1 and 15, daily 6 mg/m^2 of cisplatin for 20 administrations, and 60 Gy of HART during a 4-week treatment period. This ensured a much higher dose intensity than the protocol by Schaake-Koning et al.,[2] as theirs did not contain CPT-11 and incorporated a 3-week radiotherapy split during the treatment course.

The response rate was 50% (six patients with a partial response among 12 total patients); survival was moderate with a MST of 10.1 months, a 1-year survival rate of 50%, and a 5-year progression-free survival rate of 17% (2 of 12). Although the response rate seemed disappointing, evaluation of the tumor response after radiotherapy or chemoradiotherapy is sometimes difficult because of fibrotic pulmonary lesions caused by irradiation. In fact, two patients, cases 5 and 10, for example, enjoyed 5-year progression-free survival, in spite of their tumor response of stable disease (SD), suggesting that their lesions after therapy might not have contained viable cells although the tumor size was unaltered. The present protocol, as well as that of Schaake-Koning et al,[2] is characterized by the daily administration of low-dose cisplatin with concurrent radiotherapy. The radiosensitizing activity of cisplatin might have played some role in this result. That is, when Schaake-Koning et al.[2] demonstrated the superiority of the concurrent multimodality consisting of cisplatin and radiotherapy, the daily administration of cisplatin was more advantageous than its weekly administration although the total dose was the same. These findings seem to suggest a supra-additive effect of cisplatin when combined with radiotherapy. In fact, similar protocols utilizing daily low-dose cisplatin (ranging from 5 to 10 mg/m^2) combined with radiotherapy have been reported to be effective in NSCLC[34–37] and other types of cancer.[38–40]

Although the supra-additive effect of cisplatin combined with irradiation has been shown in many in vitro studies,[24–27] the cisplatin doses in those studies were usually high. Therefore, interpretations of such preclinical studies may not be relevant to specific situations in which daily low-dose regimens of cisplatin are used. From the pharmacokinetics analysis of cisplatin and CPT-11 included in the present study, the pharmacokinetics of CPT-11 did not seem to have significantly interfered with cisplatin, as the values obtained on days 1 and 15 were comparable. As for the cisplatin pharmacokinetics, free platinum had a similar maximum concentration (Cmax) and dropped below the lowest detection level by 24 h after every administration. A cumulative effect was not observed with free platinum. In contrast, Cmax of total platinum accumulated from 266 ± 135 ng/ml at day 1 to 1020 ± 109 ng/ml at day 26 (Fig. 1). Other pharmacokinetics studies of daily low-dose or continuously infused cisplatin also revealed an accumulation of

total platinum but not of free platinum.[41,42] In addition, the final concentration of free platinum amounted to approximately 25% of all platinum compounds, by the daily low-dose administration in this protocol, in contrast to conventional single-dose cisplatin administration, which usually yields approximately 50% free platinum in plasma, at a level near Cmax. Most of the discussion on the pharmacokinetics and -dynamics of this agent, however, have focused on this conventional method. These ostensibly different regimens may lead to different consequences in terms of activity of the agent. The way in which these factors, that is, the significantly higher ratio of the protein-bound platinum and the significantly long-lasting cumulative total platinum, might influence the antitumor activity, radiosensitizing ability, and toxicity of the agent needs to be further investigated in preclinical studies.

In conclusion, the recommended dose of CPT-11 on days 1 and 15 was defined as 40 mg/m^2 when combined with daily 6 mg/m^2 of cisplatin administered 5 days a week for 4 weeks and HART of 60 Gy in 40 fractions (twice daily) over the same period. This therapeutic regimen resulted in a 50% response rate and a MST of 10.1 months, with two patients being 5-year progression-free survivors, out of the 12 patients enrolled. Phase II studies might be warranted to clarify the activity of this regimen. In addition, further preclinical investigations will be required to clearly demonstrate the antitumor activity, including the radiosensitizing ability and toxicity of repeated administrations, of low-dose cisplatin. The present pharmacokinetics data should provide useful information for such studies.

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