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

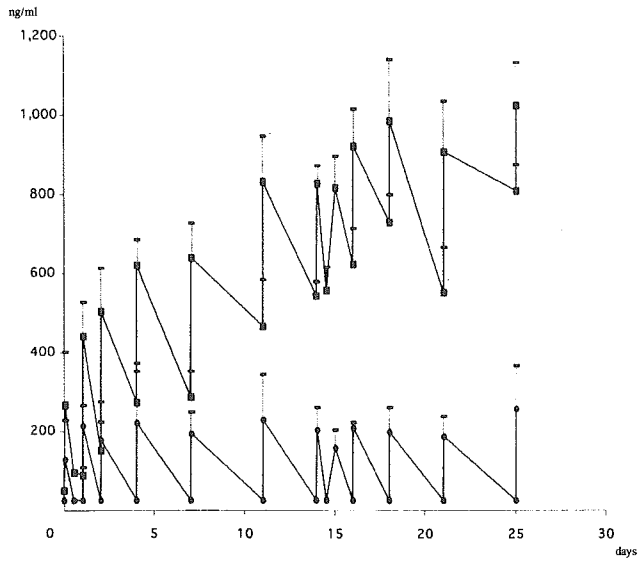

Fig. 1. Mean concentrations of free and total platinum with daily (5 days a week) administration of 6 mg/m^2 of cisplatin for 4 weeks ($n = 6$). The *dotted vertical lines* beyond the means represent standard deviations. Free platinum (*circles*) reached its maximum concentration with every administration 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

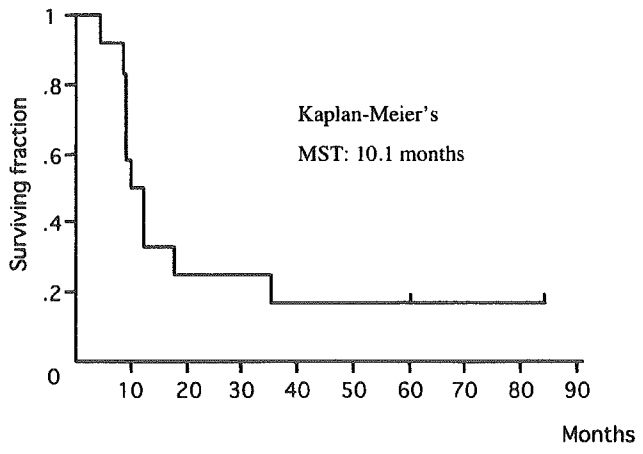

Fig. 2. The intent-to-treat survival curve (Kaplan-Meier's method) revealed 10.1 months of median survival time (*MST*) with two patients being 5-year progression-free survivors out of the 12 patients enrolled

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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Photodynamic Therapy for Cervical Intraepithelial Neoplasia

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Key Words
Human papilloma virus · Excimer dye laser · YAG-OPO laser · Photofrin · Cervical cancer

Abstract
Objectives: Photodynamic therapy (PDT) is a minimally invasive treatment for cervical intraepithelial neoplasia (CIN). We report the effectiveness of PDT in 105 cases of CIN. ***Methods:*** All patients received photofrin (PHE) 2 mg/kg intravenously and, 48–60 h later, phototherapy was performed using the Excimer dye laser or a YAG-OPO laser with an irradiation dose of 100 J/cm^2 using 630 nm wavelength. ***Results:*** Mild photosensitivity occurred in 48% (50/105) of patients. The complete response (CR) rate was 90% (94/105) at 3 months following treatment. In the remaining 11 patients, 5 patients had CIN1, 2 patients had CIN2, and 4 patients had mild cytologic findings. However, in 9 of these 11 patients, CR was achieved 6 months after PDT. In 69 patients, human papilloma virus (HPV) typing was performed before and after PDT therapy. Pre-treatment, 64 of 69 patients (93%), were HPV-positive including 30 cases of high-risk HPV (43%). Testing performed 3, 6 and 12 months following PDT revealed no HPV-DNA in 75% (52/69), 74% (48/65) and 72% (41/57) of patients. At present, the median follow-up period is 636 days (90–2,232 days). In 3 patients, recurrence requiring surgical treatment was identified at 646, 717 and 895 days after PDT. ***Conclusions:*** PDT is an effective and minimally invasive treatment for CIN, which also appears to eradicate HPV infection.

Introduction

Cancer of the uterine cervix is one of the most common malignant neoplasms among women, and remains the leading female malignancy in developing countries [1]. In 1999, about 6,500 women were diagnosed with cervical cancer in Japan [2]. In the USA, approximately 13,000 women developed cervical cancer in the year 2000 [3]. Cervical intraepithelial neoplasm (CIN) is often the precursor to cervical cancer. In 70% of CIN, evidence of the human papilloma virus (HPV) is detected [4]. In cervical carcinogenesis, HPV is thought to inactivate the cell cycle regulators by inhibiting p53 and pRb proteins by E6 and E7 proteins [5–7]. HPV is divided into two types: high-risk types and low-risk types. Only the high-risk HPV can efficiently inactivate p53 and pRb.

The current treatment of CIN is primarily based on the surgical excision using laser, loop electrosurgical procedure or cold knife conization technique. Unfortunately, these treatments often lead to obstetric problems such

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as cervical incompetence in young women who go on to become pregnant [8]. There is a novel alternative for the treatment of neoplasia called photodynamic therapy (PDT). PDT involves the systemic administration of a tumor-localizing photosensitizer, followed by the laser irradiation of the affected area [9–11]. Since PDT is minimally invasive with no surgical excision, it should be a cervix-sparing treatment, which may be particularly attractive to women desiring to preserve fertility. This paper presents a large series of patients with CIN treated with PDT.

In this study we expand on our preliminary report of the therapeutic effect of PDT in 31 CIN cases which suggested that PDT is effective for treating cervical dysplasia, and for the eradication of cervical HPV [12]. We now describe the effectiveness PDT in 105 cases, of CIN with a median follow-up period of 636 days.

Material and Methods

Patients

Between December 1996 and April 2004, 105 nonpregnant women with a histological diagnosis of CIN (CIN1: 4; CIN2: 6; CIN3: 95) were enrolled in this study. All patients hoped to retain their fertility and chose PDT for its potential as a cervix-preserving therapy. The nature and purpose of the study were fully explained to each patient, and all patients gave written informed consent. The study was approved by the institutional review board of Hyogo Medical Center for Adults and Osaka City General Hospital.

PDT

All patients received intravenous PHE, 2 mg/kg to photosensitize the lesions (Photofrin, Japan Wyeth Lederly , Tokyo, Japan). Phototherapy was performed using an Excimer dye laser (EDL) or a YAG-OPO laser (Ishikawajima-Harima, Heavy Industry, Tokyo, Japan) with an irradiation dose of 100 J/cm^2 using a 630-nm wavelength. The laser instruments were mounted on a colposcope with an optical path for the laser. For the endocervix, a specially designed intracervical probe was used. After treatment, all patients were hospitalized in a dark room with protection from light for 3 weeks. For the first week post-treatment, light was limited to 5–20 Lx, for the second week light was limited to 30–50 Lx, and for the third week light was limited to 50–100 Lx. The light was measured using a luxmeter. The patients were examined every 3 months after PDT treatment. The clinical effect was judged using cytology and directed biopsy. The primary responses were determined 3 months later after PDT. When these examinations showed no abnormal findings, the case was considered a complete remission (CR). Minor response (MR) indicates mild histological change indicating low or high grade squamous intraepithelial lesion (LSIL, HSIL) less severe than the primary disease, and partial response (PR) indicates mild cytologic findings indicating LSIL or HSIL without histologic change. Toxicity was determined using NCI-CTC ver2.

Detection of HPV

The cervical smears were collected with a cotton-tipped swab and preserved in the phosphate buffer at –80°C until analyzed. DNA was analyzed following the PCR-based methods previously described by Yoshikawa et al. [13] and Nagano et al.[14]. Briefly, samples were analyzed by L1 consensus primers for amplification and detection of HPV-DNA, and digested with *Rsa*I, *Dde*I, *Hae*III, *Hin*fI, *Xba*I, *Acc*I, *Pst*I and *Kpn*I for HPV typing by restriction fragment length polymorphism (RFLP). HPV-DNA types 16, 18, 31, 33, 51, 52, 56, 58, 61, 70 and 82 were determined with the sensitivity of 0.01–0.1 copy/cell.

Results

Clinical Response

A total of 105 women enrolled in this study. The clinical characteristics of the patients are shown in table 1. Median age is 30 years (range: 19–49). Forty-eight patients were single, 50 patients were married and 7 patients were divorced. Seventy-four patients were nulliparous, and 31 patients were multigravida. Four patients had CIN1, 6 patients had CIN2 and 95 patients had CIN3. Toxicity was predominantly mild cutaneous photosensitivity (grade 1–2 in 49 patients, and grade 3–4 in 1 patient). Grades 1 and 2 cutaneous photosensitivity were cured within 2 weeks without any treatment. One patient suffered from grade 3 photosensitivity, because she worked in the sunshine during the summer season just after being discharged. She was cured with topical steroid treatment. Minimal vaginal discomfort and discharge was also described by some patients. Cervical stenosis occurred in 11 patients. PDT was performed safely for all the patients.

The CR rate was 90% (94/105) 3 months following PDT. In the remaining 11 patients, 5 patients had CIN1, 2 patients had CIN2, and 4 patients had mild cytological findings. However, in 9 of these 11 patients, CR was achieved 6 months following PDT. In contrast, 5 patients had newly detected disease after 6 months including 3 CIN1 lesions and 2 cases of mild cytological findings. For 76 patients, the 1-year follow-up results were as follows: 2 patients had CIN1, 2 patients had mild cytological findings and 72 patients achieved CR (95%: 72/76). In 15 CR cases, cervical cytology and biopsy were performed every 3 days after PDT for 2 weeks, and within 3 days of laser treatment, necrosis of the CIN region occurred and atypical cells disappeared in all cases.

At present, the median follow-up period is 636 days (range: 90–2,232 days). In 3 patients, recurrence occurred at 646, 717 and 895 days after treatment. Two of these

Table 1. Clinical course in all cases

Patient	Age	MA	PRG	DEL	HIS	PS	HPV PRE	HPV 3M	HPV 6M	HPV 12M	HPV 24M	Effect 3M	Final prognosis	Duration
1	27	1	0	0	CIN3	G1	NEG	NEG	NEG			CR	NED	1,253
2	35	1	4	1	CIN3	G1	16+OT	NEG	52	52+OT	NEG	CR	NED	1,361
3	29	1	0	0	CIN3	G1	16	NEG	OT	OT	OT	CR	NED	1,409
4	33	1	4	0	CIN3	G1	16	NEG	NEG	NEG	NEG	CR	NED	1,244
5	37	1	2	1	CIN3	G0	16	NEG	NEG	NEG	NEG	CR	NED	1,395
6	28	1	4	1	CIN3	G1	16	NEG	NEG	NEG		CR	NED	567
7	29	1	1	1	CIN3	G1	16	NEG	NEG	NEG	NEG	CR	NED	1,255
8	26	1	1	1	CIN3	G1	16	OT	61	51	NEG	CR	NED	1,293
9	20	0	1	0	CIN3	G0	51	NEG	NEG	NEG	NEG	CR	NED	721
10	32	0	0	0	CIN2	G1	16	NEG	NEG	NEG	NEG	CR	NED	1,099
11	28	0	4	2	CIN3	G1	18	NEG	NEG	NEG	NEG	CR	NED	1,104
12	29	1	1	0	CIN3	G1	16	NEG	NEG	NEG	NEG	CR	NED	1,103
13	30	1	0	0	CIN3	G0	61	NEG	NEG	NEG	NEG	CR	NED	1,140
14	32	1	0	0	CIN3	G1						CR	NED	1,140
15	25	0	4	0	CIN3	G0	53+OT	NEG	NEG	NEG	NEG	CR	NED	1,116
16	33	1	6	2	CIN3	G0	52	NEG	NEG		NEG	CR	NED	1,011
17	38	2	1	0	CIN3	G1	58	NEG	NEG	NEG	NEG	CR	NED	1,060
18	30	1	2	1	CIN3	G1	58	NEG	NEG	NEG	OT	CR	NED	810
19	36	0	0	0	CIN3	G0	35	NEG	16	OT	NEG	CR	NED	1,024
20	30	1	1	1	CIN3	G0	52	NEG	NEG	NEG	NEG	CR	NED	917
21	30	1	1	1	CIN3	G1	16	NEG	NEG	NEG		CR	RE	717
22	25	0	0	0	CIN3	G1	16	NEG	OT	NEG		CR	NED	372
23	33	0	0	0	CIN3	G0	52+OT	OT	NEG		52	CR	NED	919
24	19	0	1	0	CIN3	G0	70+OT	70	NEG	NEG	OT	MR	NED	970
25	33	1	0	0	CIN3	G0	31	31	OT	NEG	OT	PR	RE	895
26	40	1	0	0	CIN3	G1	16+58	OT	OT	OT	OT	CR	NED	913
27	35	2	3	1	CIN3	G1	16	51	51	NEG	NEG	CR	NED	902
28	37	1	4	0	CIN3	G1	NEG	NEG	NEG	NEG	NEG	CR	NED	902
29	35	2	3	1	CIN3	G0	52+OT	NEG	NEG	NEG	58	MR	NED	810
30	42	0	1	0	CIN3	G0	82	16	NEG	16	16	CR	NED	893
31	29	1	2	1	CIN2	G1	52	NEG	NEG	NEG		CR	NED	803
32	28	0	0	0	CIN3	G1	OT	NEG	NEG	NEG	NEG	CR	NED	886
33	28	1	0	0	CIN3	G0	58	NEG	NEG	NEG		CR	NED	733
34	39	1	1	0	CIN3	G0	16	OT	NEG	NEG	OT	CR	NED	756
35	29	1	1	1	CIN3	G0	OT	NEG	NEG	NEG	OT	MR	NED	620
36	30	1	1	1	CIN3	G0	82	NEG	NEG	NEG		CR	NED	727
37	31	1	0	0	CIN3	G0	51	NEG	NEG	NEG		MR	NED	557
38	33	1	3	2	CIN3	G0	51	NEG	NEG	51		CR	NED	594
39	26	1	1	0	CIN3	G0	NEG	NEG	OT	NEG		CR	NED	371
40	23	0	0	0	CIN3	G0	51	NEG	NEG		16+53	CR	NED	636
41	36	1	0	0	CIN3	G1	16+35	NEG	NEG	NEG		CR	NED	698
42	26	0	3	0	CIN3	G0	58	NEG	NEG	NEG		CR	NED	558
43	23	0	0	0	CIN2	G0	OT	52	52+OT	54		CR	NED	547
44	33	0	0	0	CIN3	G0	51	NEG	NEG	NEG		CR	NED	568
45	25	0	0	0	CIN3	G0	16	52	52	52		CR	NED	529
46	28	0	0	0	CIN3	G0	58	NEG	NEG	NEG		CR	NED	529
47	30	2	2	1	CIN3	G1	OT	34	NEG	16		CR	NED	529
48	29	0	4	0	CIN3	G1	31	NEG	OT	NEG		CR	NED	374
49	27	0	0	0	CIN3	G0	16	NEG	NEG	OT		CR	NED	286
50	34	0	0	0	CIN2	G1	16	NEG		68		CR	NED	395
51	37	1	1	0	CIN3	G1	16	NEG	NEG	NEG		CR	NED	381
52	31	0	3	0	CIN3	G0	18	18	18	18		CR	NED	381
53	37	1	1	1	CIN3	G1	16	NEG	NEG	NEG		CR	NED	371
54	32	1	2	0	CIN3	G0	16	NEG	NEG	NEG		CR	NED	371
55	30	0	0	0	CIN3	G0	52	OT	51	68+OT		CR	NED	359

Table 1 (continued)

Patient	Age	MA	PRG	DEL	HIS	PS	HPV PRE	HPV 3M	HPV 6M	HPV 12M	HPV 24M	Effect 3M	Final prognosis	Duration
56	24	1	4	1	CIN3	G1	NEG	NEG	NEG			CR	NED	366
57	35	0	0	0	CIN3	G1	16+70	16+70	NEG	NEG		CR	NED	356
58	33	1	1	1	CIN3	G0	16	NEG	NEG			CR	NED	480
59	31	1	4	2	CIN3	G1	16	NEG	NEG	NEG		CR	NED	276
60	21	1	3	2	CIN3	G1	59	59	NEG			CR	NED	194
61	35	2	5	4	CIN3	G1	OT	NEG	NEG	OT		CR	NED	283
62	22	0	0	0	CIN1	G0	52	OT	52+OT			CR	NED	276
63	27	0	0	0	CIN3	G0	59+OT	OT	33+OT	OT		MR	NED	324
64	29	1	0	0	CIN1	G0	58	NEG	NEG			CR	NED	352
65	26	0	0	0	CIN3	G0	NEG	NEG	NEG			CR	NED	269
66	21	0	1	0	CIN3	G1	16	NEG	OT			CR	NED	273
67	33	1	0	0	CIN3	G0	16	NEG	NEG			CR	NED	269
68	24	0	0	0	CIN3	G1						CR	MC	269
69	29	0	3	1	CIN3	G1						MR	NED	269
70	21	0	0	0	CIN3	G0						CR	NED	269
71	31	1	2	1	CIN3	G1						CR	NED	266
72	41	2	0	0	CIN3	G0						CR	NED	276
73	24	0	3	0	CIN3	G1						CR	NED	273
74	34	0	4	0	CIN3	G1						MR	MC	153
75	40	0	0	0	CIN3	G1						CR	NED	184
76	27	0	1	0	CIN3	G0						CR	NED	90
77	38	0	4	0	CIN3	G1						CR	NED	118
78	32	0	0	0	CIN1	G1						CR	NED	94
79	33	1	1	1	CIN3	G0	16	NEG		NEG		PR	RE	646
80	25	0	0	0	CIN3	G1						CR	NED	648
81	36	0	0	0	CIN3	G1						CR	NED	101
82	35	2	1	1	CIN3	G3						CR	NED	643
83	21	0	0	0	CIN3	G0						CR	NED	616
84	32	1	0	0	CIN3	G0						CR	NED	901
85	22	0	0	0	CIN3	G0						CR	NED	749
86	34	0	1	0	CIN3	G0						CR	NED	845
87	24	1	0	0	CIN3	G1						CR	NED	1,015
88	29	0	0	0	CIN3	G0						CR	NED	1,114
89	20	0	0	0	CIN3	G0						CR	NED	1,298
90	20	0	0	0	CIN3	G1						CR	NED	1,112
91	27	1	1	0	CIN3	G1						CR	NED	1,850
92	49	0	0	0	CIN3	G0						CR	NED	2,116
93	25	1	1	1	CIN3	G1						CR	NED	1,410
94	26	1	0	0	CIN3	G0						CR	NED	2,232
95	29	0	0	0	CIN3	G0	58	NEG		NEG	NEG	PR	NED	2,065
96	28	1	4	1	CIN2	G0	16	NEG		NEG	NEG	CR	NED	2,035
97	25	1	0	0	CIN3	G0						CR	NED	1,653
98	34	0	1	0	CIN2	G0						CR	NED	820
99	40	1	2	2	CIN3	G1						PR	NED	481
100	35	1	2	1	CIN3	G0						CR	NED	365
101	35	1	0	0	CIN3	G1						CR	NED	360
102	39	0	0	0	CIN3	G0						CR	NED	354
103	33	1	0	0	CIN3	G0						CR	NED	180
104	30	0	0	0	CIN1	G1						CR	NED	95
105	32	1	0	0	CIN3	G0						CR	NED	93

MA = Marriage (0: single, 1: married, 2: divorced); PRG: pregnancy times; DEL = delivery times; HIS = histology; PS = photosensitivity; HPV PRE: HPV type before treatmenrt; HPV 3M = HPV type 3 months after PDT; NEG = negative; OT = Other; Effect 3M = effect of PDT after 3 months; PR = partial response; MR = minor response; NED = No evidence of disease; MC = minor change; RE = recurrence; Duration = duration after treatment (days).

patients had CIN3, and 1 patient (case 25) developed stage IB1 cervical cancer. All 3 patients required surgical intervention. Two additional patients had mild cytological changes. Of all 105 patients, 14 patients have become pregnant following PDT including 6 women who have delivered term babies without complications. The outcomes of the other 8 pregnancies include: 1 preterm delivery, 1 spontaneous miscarriage, 2 therapeutic abortions, 1 molar pregnancy, and 3 ongoing pregnancies. All clinical histories are summarized in table 1.

HPV

HPV typing was performed before and after PDT therapy for 69 patients. Before treatment, HPV was detected in 64 of 69 patients (93%), including 30 patients with high-risk HPV (16, 18). Three months after PDT, HPV-DNA could not be detected in 47 of 64 patients (73%) who showed HPV-DNA positive cervical smears before treatment. Seventeen patients still had HPV-DNA positive cervical smears, and in 13 of these 17 cases, HPV typing changed. Six months after PDT, 17 of 65 (26%) examined patients still had HPV-DNA positive cervical smears; however, these 16 patients had no abnormal cytological or histological findings. Additionally, in 15 of these 16 HPV-DNA positive cases, HPV typing changed compared to pre-PDT testing. One year after PDT, 16 of 57 (28%) examined patients still had HPV-DNA in cervical smears. Of these 16 patients, only 1 patient had mild abnormal cytological findings indicating LSIL. Additionally, in 13 of 16 HPV-DNA-positive patients, HPV typing changed compared to pre-PDT testing. Two years after PDT, 11 of 31 (35%) examined patients still had HPV-DNA in cervical smears. In 10 of 11 HPV-DNA positive patients, HPV typing changed compared to pre-PDT testing. Finally, 3 patients had recurrence (2 cases: CIN3; 1 case: invasive cancer); however, these 3 patients had negative HPV-DNA in cervical smears 1 year after PDT treatment.

Discussion

In this study, we examined the effect of PDT on CIN and HPV in over 100 women. We found that over 90% of patients achieved CR after 3 months. Only three patients ultimately developed recurrent disease. There are several studies reporting disappointing results using PDT for the treatment of CIN [15–20]; however, these investigators used 5-aminolevulinic acid for a sensitization agent which we believe is inferior to the agent used in our study. For example, Hillemanns et al. [16] performed PDT using 5-aminolevulinic (5-ALA) for sensitization and an argon-ion-pumped dye laser in 7 women with high grade CIN. However, PDT did not appear to be effective in all patients. Keefe et al. [18] performed PDT using 5-ALA and argon-pumped dye laser in 40 CIN2 or 3 patients, and reported success rates at 4, 8 and 12 months were 51, 46 and 31%. Barnett et al. [19] reported that the response rate of PDT using 5-ALA was 33% in CIN1/2 patients. These reports suggest that PDT using 5-ALA is not effective for CIN. We achieved much better response when treating CIN with PDT using PHE for photo sensitization, and an ELD or YAG-OPO laser. In addition, PHE is reported to be more effective for cascular endothelial cell than 5-ALA [21, 22]. Muroya et al. [23] performed PDT using PHE and EDL for 56 patients (39 CIS and 17 dysplasia), and achieved high complete response rate comparable to our current study (96.4%, 54/56). However, in the Muroya report, follow up duration was short and HPV typing was not evaluated. Di Saia and Creasman [24] reported that the surgical treatment including cold-knife excision, electrocautery, cryosurgery and laser ablation achieved high success rates between 90 and 98%. Recurrence rate of conization for CIN was reported to be 0.6% [25–27]. From these findings, PDT may be somewhat inferior to surgical treatment for CIN. A comparative study is needed to solve this problem.

It is well known that HPV is the most prevalent etiologic agent in neoplastic transformation of squamous epithelial cells. Cervical carcinogenesis is related to specific high risk types of HPV, most commonly HPV 16 and 18. In our series, HPV was detected in 64 out of 69 patients (93%) and high-risk HPV (16, 18) detected in 30 (43%) patients before treatment. Finally, HPV-DNA was not detected in 75, 74 and 72% at 3, 6 and 12 months after PDT. These data are consistent with Wierrani et al.'s [17] report of 19 patients undergoing PDT. One year after PDT, 16 of 57 (28%) patients in our study still had HPV-DNA in cervical smears. Of these 16 patients, only 1 patient had mild abnormal cytological findings. Additionally, in 13 of 16 HPV-DNA-positive patients, HPV typing changed compared with testing before PDT. This suggests that the 13 patients might have been re-infected with other types of HPV since treatment. Furthermore, the 3 patients with recurrent pre-invasive or invasive disease had no HPV-DNA detected in cervical smears 1 year after PDT. Persistent HPV infection did not predict the recurrence of CIN. In fact, 16 patients with HPV persistence or re-infection had no recurrence of CIN during the follow-up period.

The follow-up period in our study is too short to determine the long term effectiveness of PDT for CIN treatment. Ylitalo et al. [28] reported that among HPV 16-positive women, the median incubation period from infection to carcinoma in situ was 7–12 years. In our study, 3 patients experienced recurrence despite negative HPV testing 1 year after PDT. Possibly, our HPV detection system may have lacked sensitivity in these cases. Alternatively, not only HPV infection, but also the status of the immune system, abnormality of cell cycle regulators, and p53 polymorphisms may contribute to the development cervical neoplasia [4, 29–31]. However, given the known lengthy incubation period of neoplasia, we speculate that these recurrent cases might be due to small undetectable lesions of CIN that persisted following PDT. Further study is needed to better understand the cases that are not cured by PDT.

In this study, all patients were hospitalized to ensure light deprivation for 3 weeks. With this rigorous light-deprivation protocol, 50 of 105 patients (48%) developed mild cutaneous photosensitivity (grades 1 and 2). Only one patient suffered from grade 3 cutaneous photosensitivity. It may be because she worked in the sunshine during the summer season just after being discharged. In other reports of PDT using 5-ALA, cutaneous photosensitivity was not reported despite light exposure [15–20]. However, we believe the superior therapeutic profile of PHE justifies its use despite the increased phototoxicity. We recognize that our protocol may be prohibitively expensive and inconvenient in many settings. Since toxicity was minimal, more liberal protocols of light deprivation may be appropriate. It was reported that lower doses of PHE such as 1 mg/kg was effective for cutaneous cancer [32–33]. Decreasing the PHE dose may reduces cutaneous photosensitivity. Establishing an outpatient protocol is one of our goals for future study.

In conclusion, PDT is an effective treatment for CIN, and for HPV infection. PDT may be an attractive alternative for women desiring to preserve cervical function for pregnancy. Furthermore, in our study, the persistence of HPV following treatment did not correlate well with CIN recurrence.

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S. Yamaguchi and H. Tsuda have contributed equally to this study.

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Is laser conization adequate for therapeutic excision of adenocarcinoma *in situ* of the uterine cervix?

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Abstract

Aims: To determine the safety of uterine-preserving operations for adenocarcinoma *in situ* of the cervix.

Methods: Fifteen cases of adenocarcinoma *in situ* (AIS) were diagnosed using neodymium:yttrium aluminum garnet (Nd:YAG) laser conization. The accuracy of preconization histology or cytology was evaluated in 15 AIS cases. In these AIS cases, we investigated how far the tumor was located from the squamocolumnar junction (SCJ) and the endocervix. Fourteen cases of the 15 AIS-affected patients were treated using laser conization alone. These patients were closely followed up.

Results: Precise agreement between preconization diagnosis and conization histology was seen in 46.7% (7/15) of the AIS cases. In 14 of the 15 cases of AIS (93.3%), the tumor was adjacent to the transitional zone, within 3 mm of the SCJ, and in the other case (6.7%), the tumor was between 0 and 5 mm away from the SCJ. In all subjects, cone height was 8–18 mm (mean 13.1 mm). None of the 15 patients showed any recurrence of AIS during follow up ranging from 15 to 75 months (43.1 months on average).

Conclusions: Women with AIS who want to preserve their fecundity might be treated with laser conization alone.

Key words: adenocarcinoma *in situ*, laser conization, squamocolumnar junction, uterine cervix, uterine preservation.

Introduction

The incidence of uterine cervical adenocarcinoma among cervical cancers has been reported to be 5–9%,[1–4] or 5–15%;[5] thus, uterine cervical adenocarcinoma is assumed to be relatively rare. Cervical adenocarcinomas occur between the squamocolumnar junction (SCJ) and the internal os and are thus difficult to observe directly. Consequently, cervical adenocarcinoma might be missed in its early stages.

Even when a lesion is discovered, although it is still considered a microinvasive adenocarcinoma, a radical operation, including lymphadenectomy is frequently carried out because cervical adenocarcinomas are more aggressive than squamous cell carcinomas, and the prognosis is usually poor.[6] Thus, although the uterus often can be preserved in the treatment of early squamous cell carcinoma, preservation of the uterus is more difficult in cases of cervical adenocarcinoma. Some authors have reported that young women with cervical adenocarcinoma *in situ* (AIS) who want to preserve their fertility might be treated with a conservative procedure, such as conization of the uterine cervix if the surgical margins are free of cancer.[7–10] However,

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there is no consensus as to whether conization is adequate treatment for AIS because residual foci of tumor might remain and present subsequently as invasive adenocarcinoma.[11–13]

Comparing the diagnosis of pre- with post-cone biopsy, the accuracy of diagnosing AIS using cytology and punch biopsy also was evaluated in the present study. Furthermore, AIS patients treated at our hospital were reviewed retrospectively to determine the advisability of preserving the uterus in cases of AIS.

Patients and Methods

Data from 15 patients with AIS of the cervix, as the final pathologic diagnosis treated at our hospital between 1989 and 2002, were reviewed for this study. All patients underwent laser conization of the uterine cervix. The same contact Nd:YAG laser conization procedure was carried out in all 15 patients. In summary, the excision was carried out with an output of 25 watts and at the endocervical margin, the cone was transected with scissors. The endocervical curettage was carried out in postoperative registration, and the cervical canal was always examined. All conization specimens were cut longitudinally. After being fixed at room-temperature in 10% formalin for 16–40 h, the cone specimens were step-sectioned by radical cuts, and the blocks were paraffin-embedded with sections cut at 3 μm and stained with hematoxylin and eosin. The mean number of blocks was 12.7 (range 8–24). The hospital's pathologist diagnosed all cases. In eight cases, the preoperative diagnosis was CIN3 or microinvasive carcinoma. In seven cases, laser conization was carried out because atypical glandular cells were present in cervical smears or AIS was identified in punch biopsy specimens. All patients were informed the risk of cervical adenocarcinoma if they underwent only laser conization.

The diagnosis before conization was compared with the pathologic diagnosis of the conization specimens. In the 15 cases where the pathologic diagnosis of the conization specimen was AIS, we measured the vertical distance of the cervical region domain between the SCJ and the distal edge of the tumor. The mean age of the AIS patients was 36.3 years. In the one case of AIS that was diagnosed using laser conization, an abdominal hysterectomy with lymphadenectomy was carried out. Postoperatively, all patients were followed up with cytologic and colposcopic examination every 3–4 months for as long as possible.

Results

Among the eight cases where the preoperative diagnosis was CIN3 or microinvasive carcinoma, all conization specimens contained AIS. Of the 15 cases of AIS diagnosed postoperatively (cases 1–15), only seven (46.7%) were diagnosed accurately. Thirteen of the 15 women with AIS strongly wanted to preserve their fecundity and one case (no. 5) did not want the sequential hysterectomy. These women underwent only conization with close follow up. The remaining

Table 1 The outcome of conization for adenocarcinoma *in situ* (AIS) of the cervix

Case no.	Age (years)	Punch biopsy	Final diagnosis	Margin status	Follow up (months)	Recurrence
1	37	AIS	AIS + CIS	Cancer is free	75	(–)
2	29	AIS + CIS	AIS + CIS	Cancer is free	67	(–)
3	40	SD	AIS + SD	Cancer is free	70	(–)
4	35	AIS + CIS	AIS + CIS	Cancer is free	36	(–)
5	49	CIS	AIS + CIS	Cancer is free	68	(–)
6	33	CIS	AIS + CIS	Cancer is free	57	(–)
7	44	CIS	AIS + CIS	Cancer is free	52	(–)
8	38	AIS + CIS	AIS + CIS	Cancer is free	17	(–)
9	35	AIS	AIS + SD	Cancer is free	51	(–)
10	41	AIS	AIS + SD	Cancer is free	15	(–)
11[†]	36	CIS	MIC + AIS	Cancer is free	48	(–)
12	39	MIC	MIC + AIS	Cancer is free	32	(–)
13	32	CIS	AIS + CIS	Cancer is free	21	(–)
14	31	AIS + SD	AIS + CIS	Cancer is free	19	(–)
15	26	CIS	AIS + CIS	Cancer is free	18	(–)

[†]Patient no. 11 underwent sequential radical hysterectomy. Neither residual tumor nor lymph node metastasis was found in the hysterectomy specimen. CIS, carcinoma *in situ*; MIC, microinvasive carcinoma; SD, severe dysplasia.

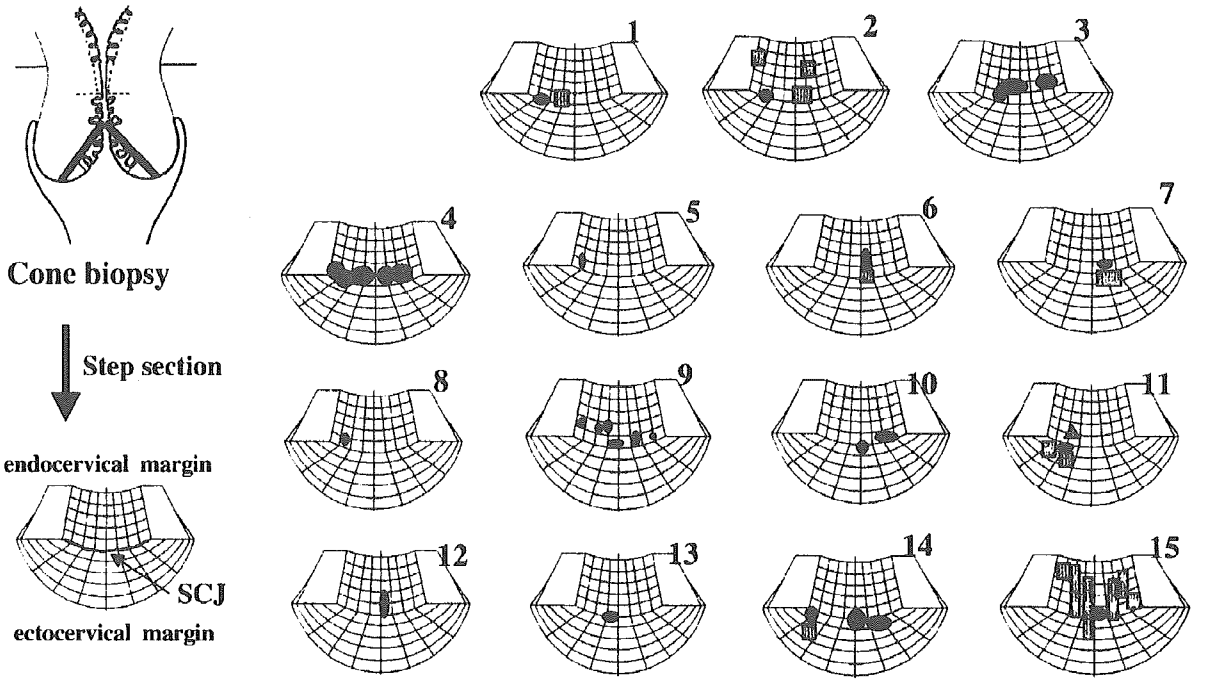

Figure 1 Schematic representation of the location of (●) adenocarcinoma *in situ* (AIS), (■) carcinoma *in situ* (CIS) and (▲) microinvasive carcinoma (MIC) in conization specimens. Conization specimens were divided into 8–24 blocks. Case numbers 3, 4, 5, 8, 9, 10, 12 and 13 contain neither CIS nor MIC in conization specimens. SCJ, squamocolumnar junction.

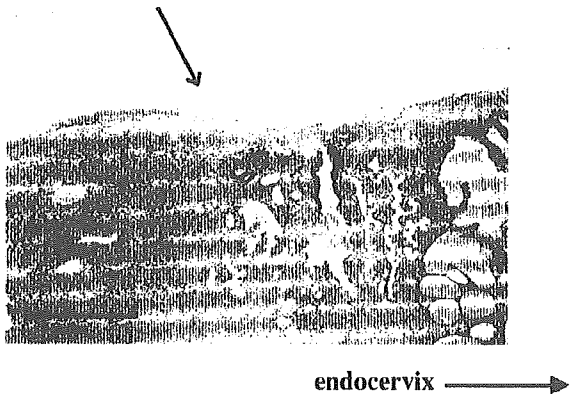

Figure 2 Relation between adenocarcinoma *in situ* (AIS) and the squamocolumnar junction (SCJ; →). AIS exists in the surface of the uterine cervix. Normal cervical glands are seen in the deeper layers (hematoxylin and eosin, ×80).

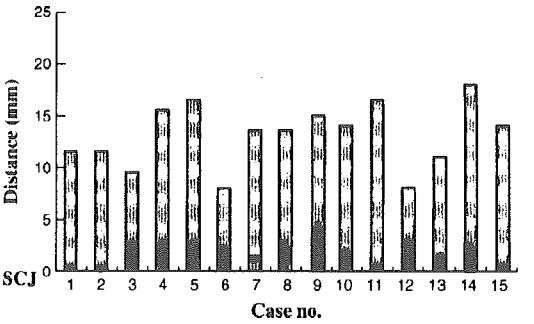

Figure 3 Distance between the squamocolumnar junction (SCJ) and the surgical margin in patients with adenocarcinoma *in situ* (AIS) who underwent conization. AIS tumor was localized adjacent to SCJ. The surgical margins were free of cancer in all cases. (●) Distance between both tumor edges.

patient underwent hysterectomy and showed no cancerous lesions (Table 1). In all 15 patients, the locus in all cases except one was adjacent to the transitional zone, within 3 mm of the SCJ (Figs 1–3). In the remaining case, the tumor was 0–5 mm from the SCJ. Cone height of conization specimens in all patients was 8–18 mm (mean 13.1 mm). Four of the subjects became pregnant during the follow-up period. No patient suffered a recurrence. An abdominal operation was carried out in one subject.

Discussion

The absence of recurrences among women with AIS after laser conization suggests that this modality is safe and effective for the treatment of these lesions.

If the surgical margin is always clear in conization specimens containing AIS, conization of the uterine cervix alone might be curative for AIS. However, AIS is difficult to diagnose using Papanicolaou smears, and the reported incidence is only 0.0002–0.004%.[5,14–18] Furthermore, AIS is difficult to diagnose in biopsy specimens because it does not always exhibit intraepithelial abnormalities during a colposcopy.[18,19] In this study, of 15 AIS cases, only seven were diagnosed as AIS preoperatively (46.7%; Table 1). In contrast, of 11 cases diagnosed or suspected as AIS preoperatively, four cases were diagnosed as having a microinvasive adenocarcinoma (MIAC) by laser conization procedure. Thus, conization of the cervix and histopathologic confirmation is essential for a definitive diagnosis of AIS. Some authors have stressed that conization of the cervix is not adequate treatment for AIS because the entire cervical gland area, where adenocarcinoma could occur, cannot be removed.[11,20,21] Azodi *et al.* reported that cervical adenocarcinomas are occasionally missed and that one of 16 cases of AIS (6.3%) thought to have been resected completely by conization had residual tumor tissue in subsequent conization or hysterectomy specimens.[20] Other authors have reported that when conization was carried out for AIS, with clear surgical margins, residual tumors have been found in 0–44% of surgical specimens when subsequent hysterectomies were carried out.[21–23] Thus, evidence exists that conservative treatment will cause incomplete resection in some cases.[11,12]

With regard to the possibility of incomplete resection, Lea *et al.* reported that endocervical curettage (ECC) is useful for detecting the presence of residual AIS.[24] In the present study, ECC was not carried out at the time of conization. Nevertheless, after conization, endocervical cytologic and colposcopic examination and ECC as needed were always carried out. We think doing so enabled early detection of residual lesions. However, the length of the cervical gland region of the conization specimens was not mentioned in those studies except one. Bertrand *et al.*[25] reported that the conization specimen should be at least 25 mm in depth and have negative margins to ensure that the patient has no residual disease and is therefore at a low risk of recurrence. In contrast, some authors state that AIS are adjacent to the SCJ. Andersen *et al.* maintained that because AIS develops in the transitional zone, conization with a clear surgical margin is adequate treatment.[18] Likewise, Teshima *et al.* reported that 90% of all AIS are located in the transitional zone or adjacent to the SCJ.[6] Of the 15 patients with AIS in this study, 14 tumors (93.3%) were within 3 mm of the SCJ. Our conization method excised at least 8 mm of the cervical gland region, so all 15 AIS surgical specimens had clear margins. Similarly, other authors have found that AIS might be treated using conization alone if the surgical margins are clear.[7–9]

It has been reported that adenocarcinomas often coexist with *in situ* squamous cell carcinomas.[6,11] All 15 of our confirmed cases of AIS coexisted with microinvasive carcinoma (stage Ia1), carcinoma *in situ* or severe dysplasia (CIN3).

The outcome of conservative treatments for AIS of the cervix is reportedly poor. Kuohung *et al.* found that AIS recurred in one of 12 patients (7%) treated with conization alone.[26] Widrich *et al.* described the outcome in 24 patients managed conservatively who had clear conization margins, two patients (8.3%) suffered a recurrence of AIS in that series.[27] In the present study, the follow-up period ranged from 15 to 75 months in patients treated with conization alone, and no patient developed recurrent disease.

In contrast, Östör *et al.* reported that the prognosis of microinvasive adenocarcinoma is similar to that of squamous cell carcinoma of the cervix and should be managed similarly.[28,29] However, other authors have emphasized that the risks of residual tumor and recurrent disease require more aggressive management.[10,30,31] We found that MIAC tended to occur relatively far from the SCJ compared with AIS.

Because AIS of the uterine cervix often occurs adjacent to the SCJ and is characterized by a low potential for lymph node metastasis,[32] women with AIS and clear surgical margins after conization might be followed without further treatment. However, radical surgery is probably advisable for patients with MIAC because MIAC often occurs at a relatively distant location from the SCJ, and conization might miss residual foci of tumor.

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