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Phase I study of daily cisplatin and concurrent radiotherapy in patients with cervical carcinoma

Akira Mitsuhashi[a,*], Takashi Uno[b], Naotake Tanaka[a], Kiyomi Suzuka[a], Shinichi Tate[a], Koji Yamazawa[a], Hideo Matsui[a], Seiji Yamamoto[b], Hisao Ito[b], Souei Sekiya[a]

[a]*Department of Reproductive Medicine, Graduate School of Medicine, Chiba University, Chiba 260-8670, Japan*
[b]*Department of Radiology, Graduate School of Medicine, Chiba University, Chiba 260-8670, Japan*

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Abstract

Objective. Chemoradiation based on cisplatin is the standard treatment for locally advanced cervical carcinoma; however, the optimal scheduling and dosing have still not been established. This study was conducted to determine the maximum-tolerated dose (MTD) of cisplatin for daily administration during pelvic radiotherapy (RT).

Methods. Fourteen patients with locally advanced cervical carcinoma and 13 who required postoperative RT were registered. A low dose of cisplatin was given daily concurrently with RT. Cisplatin dosing was started at 6.0 mg/m^2/day, which was incremented by 0.5 mg/m^2/day. RT was delivered at 2 Gy/day to a total dose of 50 Gy. The MTD was defined as the dose level immediately below that causing dose-limiting toxicity (DLT) in over one-third of treated patients.

Results. Twenty-five patients were treated with a maximum of six escalating dose levels. In 22/25 patients (88%), cisplatin was administered continuously as planned without interruption. The MTD was determined to be 8 mg/m^2 and the DLT was indicated by the onset of neutropenia.

Conclusion. Daily cisplatin, at 8 mg/m^2/day, is a well-tolerated radiosensitizer in cervical carcinoma patients.

Keywords: Cervical carcinoma; Phase I; Cisplatin; Chemoradiation

Introduction

Cervical carcinoma is the most frequent cause of death by cancer in women worldwide [1]. Radiation therapy is considered to be the gold standard of treatment for stage IIB–IVA patients. Recently, several phase III studies showed that concurrent chemoradiation could improve outcomes more than radiotherapy alone [2–6]. Cisplatin and cisplatin plus 5-fluorouracil have been the two most common radiosensitizer regimens used in cervical cancer. However, the Gynecologic Oncology Group 120 study showed that 40 mg/m^2 of cisplatin weekly for 6 weeks was as effective as, yet less toxic than, a combination of cisplatin plus 5-fluorouracil. Thus, weekly 40 mg/m^2 cisplatin with concurrent radiotherapy seems to have the better therapeutic ratio [5]. Although the new paradigm of cisplatin-based concurrent chemoradiotherapy is a step forward, questions remain regarding optimal scheduling, dosing, and systemic agents.

In non-small-cell lung cancer, phase III studies demonstrated that radiotherapy combined with daily administration of 6 mg/m^2 cisplatin offered improved local control and improved actuarial survival in comparison with the radiation alone group (significantly) and the weekly administration group (not significantly) in inoperable patients [7]. Several

* Corresponding author. Department of Reproductive Medicine, Graduate School of Medicine, Chiba University, 1-8-1 Inohana Chuo-ku, Chiba 260-8670, Japan. Fax: +81 43 226 2122.
E-mail address: makira-cib@umin.ac.jp (A. Mitsuhashi).

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authors who combined high-dose radiotherapy with 6 mg/m^2 cisplatin daily did not observe either renal or severe hematological toxicity in head, neck, or non-small-cell lung cancers [8–11].

On these grounds, we thought that daily administration of cisplatin was as effective as weekly administration given concurrently with pelvic radiation in patients with cervical carcinoma. We also initiated a phase I study to evaluate the maximum tolerated dose of daily cisplatin given concurrently with pelvic radiation to patients with cervical carcinoma.

Methods

Patient selection

Fourteen patients with locally advanced cervical carcinoma and 13 who required postoperative RT were entered in this study. Eligibility criteria for postoperative radiation included the presence of at least one of the following: positive pelvic lymph node metastasis, a positive surgical margin, deep stromal invasion, and parametrium invasion. Patients with either disease outside the pelvis or para-aortic lymph node swelling were not eligible. The following were the other inclusion criteria: (1) aged ≤ 75 years; (2) ECOG performance status ≤ 2; (3) no previous chemotherapy or radiotherapy; (4) leukocytes ≥ 3000/mm^3; (5) neutrophils ≥ 2000/mm^3; (6) platelets ≥ 100,000/mm^3; (7) serum creatinine ≤ 1.5 mg/dl; (8) normal chest radiograph and electrocardiogram; and (9) informed consent.

This study was approved by the Institutional Review Board of Chiba University.

Radiotherapy

Patients were treated with 10 MV X-rays from a linear accelerator using four-field box technique, 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. Anterior and posterior borders of lateral fields were carefully determined based on the pretreatment diagnostic imaging 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 entire sacrum. A CT-simulator with three-dimensional treatment planning system was used for all patients.

No attempt was made to irradiate the para-aortic lymph node region. A total dose of 50 Gy was delivered in 25 daily fractions of 2.0 Gy, administered on 5 days a week (from Monday to Friday). All fields were treated each day. Low dose-rate brachytherapy was applied for curative cases 1–2 weeks after the end of external-beam radiotherapy. Brachytherapy was not performed in the adjuvant setting.

Chemotherapy

Each dose of cisplatin was administered i.v. over 30 min, and was completed 1 h before irradiation. The daily dose of cisplatin was reconstituted in 100 ml of normal saline. All patients received 5 mg of granisetron 1 h before cisplatin to prevent emesis. Post-cisplatin hydration was performed with 1 L of normal saline given over 2 h.

Study design

A phase I study was designed to define the MTD of daily cisplatin and pelvic radiotherapy. The starting dose of cisplatin was 6 mg/m^2/day and increments of 0.5 mg/m^2/day were planned at each level until DLT occurred. The MTD was defined as the highest safely tolerated dose with toxicity levels that did not exceed the DLT. DLT was defined as grade 3 or 4 neutropenia or thrombocytopenia and grade 3 or 4 nonhematologic toxicity except for alopecia, nausea, and vomiting. Toxicity was evaluated according to National Cancer Institute common toxicity criteria and the Radiation Therapy Oncology Group toxicity criteria. Cisplatin was suspended if grade ≥3 toxicity appeared, and was resumed once the counts rose above grade 3 levels at the dose level below that which produced DLT. Radiotherapy was suspended if grade 4 hematological toxicity appeared or in the event of grade 4 radiation-related gastrointestinal or genitourinary toxicity, and treatment was resumed once the counts rose above those levels.

The dose was escalated to the next level if none of the patients experienced DLT. If the incidence of DLT was >33% (seen in 2 or 3) at a given dose level, then dose escalation was stopped. If one of three patients at any level developed treatment-related DLT, three additional patients were then treated at the same dose level. The MTD was defined as the dose level below that which produced DLT in more than one-third of the treated patients. If DLT appeared in only one or two of the six patients, the dose was escalated to the next level.

Laboratory studies, including chemistry panels and a complete blood cell count, were obtained twice weekly, or more frequently if clinically indicated.

Chemoradiation with weekly cisplatin

From December 1999 to March 2002, 10 patients with cervical carcinoma, stages IIB–IIIB, were treated with five weekly courses of cisplatin 40 mg/m^2 during standard pelvic radiation. Radiation was administered according to the same schedule as daily cisplatin. Cisplatin was withheld in any case of grade 3 toxicity (except nausea/vomiting) until the toxicity regressed to

less than grade 3. If grade 3 neutropenia appeared, G-CSF was administered.

Results

Between April 2002 and December 2003, a total of 27 patients were enrolled in the study. (Table 1). The mean age was 51.0 (range 29–71) years. The mean BMI was 24.1 (range 16.8–30.6). Two were not eligible because of non-dose-related toxicity (grade 2 nausea/vomiting) and were refused chemotherapy (2× and 15× cisplatin). Twenty-five patients were evaluable for toxicity analysis. Six dose levels were studied (Table 2). DLT was observed in six patients: in two patients at level 3, in one at level 5 and in three at level 6 (Table 3). Thus, the MTD of daily cisplatin was defined 8 mg/m^2/day.

In 22/25 patients (88%), daily cisplatin could be administered continuously as planned with no interruption. Cisplatin administration had to be interrupted in only two patients and terminated in only one.

Hematological toxicity was mild overall. As shown in Table 3, grade 3 or 4 leukopenia or neutropenia was recorded in nine cases (including five after treatment). Only one patient was treated with G-CSF because of grade 4 leukopenia (level 6); in no case was febrile neutropenia recorded. Grade 3 thrombocytopenia was observed in one patient after treatment. No grade 3 nonhematological toxicity was seen. Four patients observed grade 2 nausea and vomiting. Grade 1 and 2 diarrhea was frequent, being recorded in almost all patients. But no grade 3 diarrhea was not found. No late toxic event was observed during follow-up of patients. There was no correlation between BMI and side effects.

Fourteen patients were receiving primary treatment and were evaluated for response. Thirteen patients achieved responses: 11 (78.6%) complete responses and 2 (14.3%) partial. At the median follow-up period of 14.2 months (range 7–26), one patient with progression had died of the disease, and two patients suffered relapses at sites outside radiation field.

Table 1
Patient characteristics

Number of patients	27
Age(years)	
Mean	51.0
Range	29–71
BMI	
Mean	24.1
Range	16.8–30.6
Histology	
Squamous	19 (8)[a]
Adeno	6 (4)[a]
Small cell	1 (1)[a]
Carcinosarcoma	1 (0)[a]
Stage	
IB	6 (6)[a]
IIB	9 (7)[a]
IIIB	11 (0)[a]
IVA	1 (0)[a]
Radiation	
Adjuvant	13
Primary therapy	14

[a] Parentheses indicate members of the adjuvant group.

Table 2
Toxicity and dose levels

Toxicity	Dose levels of cisplatin (mg/m^2/day)					
	1 (n = 5)[a]	2 (n= 3)	3 (n = 7)[a]	4 (n = 3)	5 (n = 6)	6 (n = 3)
Hematological						
Leukopenia						
1	1	1	3	1	1	0
2	2	1	0	1	3	0
3	0	0	4	0	2	2
4	0	0	0	0	0	1
Neutropenia						
1	1	1	3	1	2	0
2	2	0	2	1	3	1
3	0	0	2	0	1	1
4	0	0	0	0	0	1
Thrombocytopenia						
1	2	4	6	2	4	2
2	0	0	0	0	0	0
3	0	0	0	0	0	1
4	0	0	0	0	0	0
Nonhematological						
Nausea/vomiting						
1	3	2	3	2	4	1
2	1	0	1	0	0	2
3, 4	0	0	0	0	0	0
Diarrhea						
1	1	3	2	2	4	1
2	3	0	4	1	1	2
3, 4	0	0	0	0	0	0

[a] One patient from this group was not eligible for this study.

Table 3
Dose regimens administered, toxicity, and interruption of administration

Dose level	Dose of cisplatin (mg/m^2/day)	No. of patients with DLT[a]	DLT[a]	Interruption of cisplatin administration
1	6	0/4		
2	6.5	0/3		
3	7	2/6	grade 3 neutropenia	D-21, 23,24
			grade 3 neutropenia	D-22, 24
4	7.5	0/3		
5	8	1/6	grade 3 neutropenia	7 days after treatment
6	8.5	3/3	grade 4 neutropenia	D-24
			grade 3 neutropenia	4 days after treatment
			grade 3 thrombo-cytopenia	4 days after treatment

[a] DLT: dose-limiting toxicity.

Chemoradiation with weekly cisplatin; the mean course of cisplatin was 4.2 cycle (mean total dose 168 mg). The proportion of patients who received the total course of treatment was 30%. Grade 3 and 4 hematologic toxicity was recorded in six cases (60%): Five cases of grade 3 and one of grade 4 leukopenia/neutropenia and two cases of grade 4 thrombocytopenia. Grade 3 nonhematologic toxicity occurred in one patient.

Discussion

In the present study, we sought the MTD of daily 8 mg/m^2 administration of cisplatin given concurrently with pelvic radiotherapy in patients with cervical cancer. Neutropenia was the DLT at daily cisplatin dose level of 8.5 mg/m^2.

Cisplatin-based concurrent chemoradiation was regarded as standard treatment for locally advanced cervical carcinoma. Despite the increasing use of cisplatin to exploit its powerful radiosensitizing properties, its nephrotoxicity has been recognized as its main dose-limiting feature since its early clinical trials. Therefore, another agent, namely carboplatin, was tried as a radiosensitizer [12–14]. In this study, no patient recognized any alteration of renal function. Even if the patient has a urinary tract obstruction, as long as the serum creatinine level <1.5, daily administration of cisplatin is considered to be safe. Other authors, who combined radiotherapy with 6 mg/m^2 cisplatin daily in the treatment of lung carcinoma, observed neither renal nor severe hematological toxicity [8–11].

Daily cisplatin administration led to milder adverse side effects than weekly cisplatin. Weekly cisplatin 40 mg/m^2 was accompanied with grade 3 or 4 gastrointestinal and hematological side effects, in 14% and 28.3% of patients, respectively [4]. In our study, weekly cisplatin 40 mg/m^2 caused grade 3 or 4 gastrointestinal and hematological side effects, in 10% and 60% of patients, respectively. However, only 30% of patients received the entire course of weekly cisplatin 40 mg/m^2, and a complete course of daily cisplatin 8 mg/m^2 could be administered to Japanese women. There was no phase I study of weekly cisplatin concurrent with radiotherapy in Japanese cervical carcinoma patients. We suggest that 40 mg/m^2 of cisplatin weekly is not the optimal dose for Japanese women. With daily cisplatin (≤8 mg/m^2), grade 3 gastrointestinal side effects were uncommon and 6 of 22 patients had grade 3 or 4 hematological toxicity (27.3%). We regard the daily administration of cisplatin to be more tolerable than its weekly administration. Moreover, we considered that 8 mg/m^2 could be administered daily in an outpatient situation.

Although the evaluation of response was not the primary objective of this study, the overall response rate was higher than 90%, which suggests that this treatment is clinically relevant. However, the small sample size of this phase I study precludes any conclusions about the response. The results of the present study warrant further phase II study of cervical cancer using a daily administration of 8 mg/m^2 cisplatin concurrently with pelvic radiotherapy.

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Cytogenetic and Genome Research

G2 chromatid damage and repair kinetics in normal human fibroblast cells exposed to low- or high-LET radiation

T. Kawata,[a] H. Ito,[a] T. Uno,[a] M. Saito,[a] S. Yamamoto,[a] Y. Furusawa,[b] M. Durante,[c] K. George,[d] H. Wu,[d] and F.A. Cucinotta[d]

[a] Department of Radiology, Graduate School of Medicine, Chiba;
[b] Heavy-Ion Research Group, National Institute of Radiological Sciences, Chiba (Japan);
[c] Department of Physics, University Federico II, Naples (Italy);
[d] NASA Lyndon B. Johnson Space Center, Houston, TX (USA)

Abstract. Radiation-induced chromosome damage can be measured in interphase using the Premature Chromosome Condensation (PCC) technique. With the introduction of a new PCC technique using the potent phosphatase inhibitor calyculin-A, chromosomes can be condensed within five minutes, and it is now possible to examine the early damage induced by radiation. Using this method, it has been shown that high-LET radiation induces a higher frequency of chromatid breaks and a much higher frequency of isochromatid breaks than low-LET radiation. The kinetics of chromatid break rejoining consists of two exponential components representing a rapid and a slow time constant, which appears to be similar for low- and high-LET radiations. However, after high-LET radiation exposures, the rejoining process for isochromatid breaks influences the repair kinetics of chromatid-type breaks, and this plays an important role in the assessment of chromatid break rejoining in the G2 phase of the cell cycle.

A number of experiments have been performed to quantify the biological effects of high-LET radiation exposure and results prove that this type of radiation is more lethal to cells than equivalent doses of sparsely ionizing radiation such as γ- or X-rays (Suzuki et al., 1989; Raju et al., 1991; Napolitano et al., 1992). High-LET radiation exposures produce more chromosome breakage and more complex chromosome rearrangements, which usually leads to cell death. However, some types of damage may confer a proliferative advantage on cells leading to oncogenic cell transformation and carcinogenesis. Indeed, the frequencies of transformation and mutations induced by high-LET radiation have been shown to be greater than those induced by similar doses of low-LET radiation (Thacker et al., 1979; Yang et al., 1985; Suzuki et al., 1989; Tsuboi et al., 1992), suggesting that carcinogenesis is the most important biological effect caused by exposure to high-LET radiation.

Chromosome aberration analysis is one of the most reliable and sensitive methods of measuring radiation-induced damage. Although cytogenetic damage is typically evaluated in the mitotic phase of the cell cycle, this raises problems because many cells experience severe cell cycle delays and interphase cell death (Suzuki et al., 1990; Ritter et al., 1992, 1996; Edwards et al., 1994, 1996; George et al., 2001), especially after high-LET radiation exposure. Assessing damage in interphase chromosomes can reduce some of these problems and produce a more accurate determination of cytogenetic effects following high-LET exposure. The premature chromosome condensation (PCC) technique, first described by Johnson and Rao (Johnson and Rao, 1970), condenses interphase chromosomes by fusion to mitotic inducer cells, and this method contributed greatly to the study of early effects of radiation damage and chromosome break rejoining. However, this fusion PCC method is technically difficult to perform and laborious; the PCC index is low, and

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Request reprints from: Dr. Tetsuya Kawata, Department of Radiology (L1)
Graduate School of Medicine, Chiba University
1-8-1 Inohana Chuo-ku Chiba-shi, Chiba 260-8670 (Japan)
telephone: +81-43-226-2100; fax: +81-43-226-2101
e-mail:tkawata@med.m.chiba-u.ac.jp.

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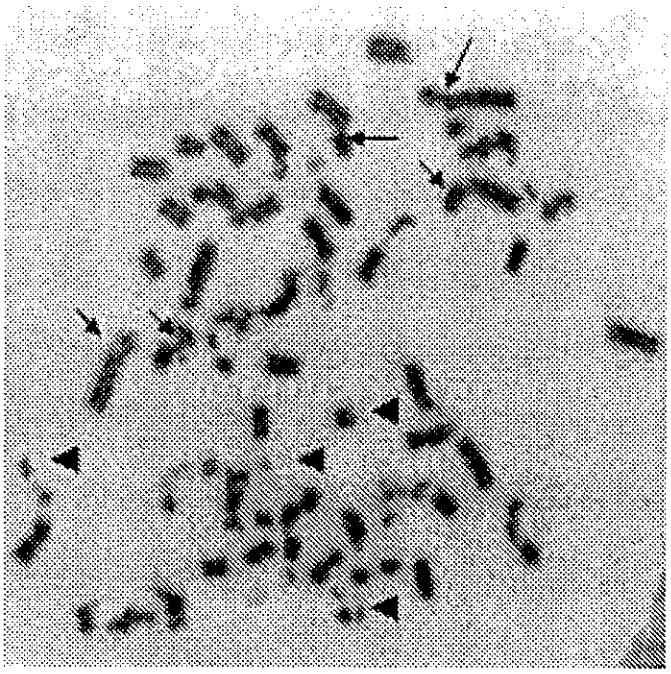

Fig. 1. An example of G2 PCC immediately after exposure to 2 Gy of 80 keV/μm carbon particles. Arrows show chromatid-type breaks and arrow heads show isochromatid breaks.

chromosomes are not well condensed. The fusion PCC technique also requires a considerable manipulation time and is therefore not amendable to studying chromosomal breaks induced immediately after irradiation.

With the recent introduction of a technique using the protein phosphatase inhibitor calyculin-A to induce condensation in interphase cells (Gotoh et al., 1995; Durante et al., 1998a), PCC collection is now technically much simpler and a higher index of well-condensed chromosomes can be obtained. Calyculin-A can induce PCC in many types of cells and in different phases of the cell cycle, especially in G2-phase cells and condensation is induced within five minutes of application. Using this technique, Durante and colleagues (Durante et al., 1999) found similar frequencies of aberrations in G2 chromosomes condensed using calyculin-A and in chromosomes condensed in G1 using the fusion PCC technique. However, lower frequencies were observed in chromosomes collected at metaphase, apparently due to the effect of cell cycle delay or cell cycle block. In this report, high-LET radiation-induced chromosome aberrations in G2-phase normal human fibroblast cells are discussed.

Initial chromatid breaks

Gotoh et al., (1999) studied radiation-induced G2 chromatid breaks using calyculin-A-induced PCC from human fibroblast cells (AG01522) after γ-ray exposure during exponential growth phase, and initial chromatid breaks were found to increase linearly with dose. Using a similar method, Kawata et al. (2000, 2001a, 2001b) studied high-LET radiation-induced G2 chromosome aberrations in AG01522 cells. An example of chromatid damage observed immediately after exposure to 2 Gy of 80 keV/μm carbon ions is shown in Fig. 1, where more than 20 isochromatid breaks (G2 fragments) and a number of chromatid breaks are observed.

The dose-response curves for chromatid-type breaks, isochromatid breaks, and total break yield (chromatid-type plus isochromatid-type) after exposure to radiation of different LET values are summarized in Fig. 2. The LET values for the radiation used here range from 0.6 to 440 keV/μm. A linear increase in chromatid breaks and total break yield was discovered, which was independent of the radiation type. On the other hand, isochromatid breaks increased linearly after exposure to high-LET radiation, and a linear quadratic increase was observed after γ-ray and 13 keV/μm carbon exposure. Interestingly, as the LET value increased, the initial percentage of chromatid-type breaks decreased and the percentage of isochromatid breaks increased, until finally isochromatid breaks predominated over chromatid-type breaks after the 440 keV/μm iron irradiation. More than 50% of the initial breaks are isochromatid-type after 440 keV/μm iron particle exposure, while more than 90% are chromatid-type breaks after γ-ray exposure.

The differences in break patterns for low- and high-LET radiations may be attributed to the structure of G2 chromosomes and densely ionizing clusters produced by high-LET radiation. In the G2 phase of the cell cycle, sister chromatids are tightly attached to one another (Murray and Hunt, 1993) and the two chromatid breaks that lead to an isochromatid break would be in close proximity. The probability of a single track of low-LET radiation producing two breaks on sister chromatids is low because ionizations are generally spaced farther apart than the distance between sister chromatids, and therefore chromatid-type breaks would predominate after low-LET exposure. However, the probability of an isochromatid break occurring from a single track of high-LET radiation is proportional to the LET of the charged particles because the distance between the ionization clusters decreases with increasing LET. An increased yield of isochromatid breaks after α-particles or neutron exposure has also been reported (Durante et al., 1994; Griffin et al., 1994; Vral et al., 2000) using mitotic collection and the G2-assay. A high percentage of isochromatid breaks can, therefore, be a signature of high-LET radiation exposure of G2 phase cells.

When the distribution of isochromatid breaks is assessed within the cell, an overdispersion is observed for high-LET exposure when compared with similar doses of low-LET radiation. Kawata et al. (2002) calculated the relative variance (s^2/y) from the measured value of the mean value (y) and the variance (s^2), which was around 1.3 for 1.2 Gy of γ-rays, 2.0 for 1.5 Gy of 185 keV/μm iron, and around 3.1 for 1.5 Gy of 440 keV/μm iron particles, respectively. Because energy deposition is focused along the high-LET particle tracks, some cells will be very heavily damaged, while cells hit by δ-rays alone will suffer modest damage, and other cells with no hits will be normal (Cucinotta et al., 1998). This is in contrast to low-LET radiation exposure, such as γ-rays, where a more even distribution of damage will induce a uniform distribution of isochromatid breaks.

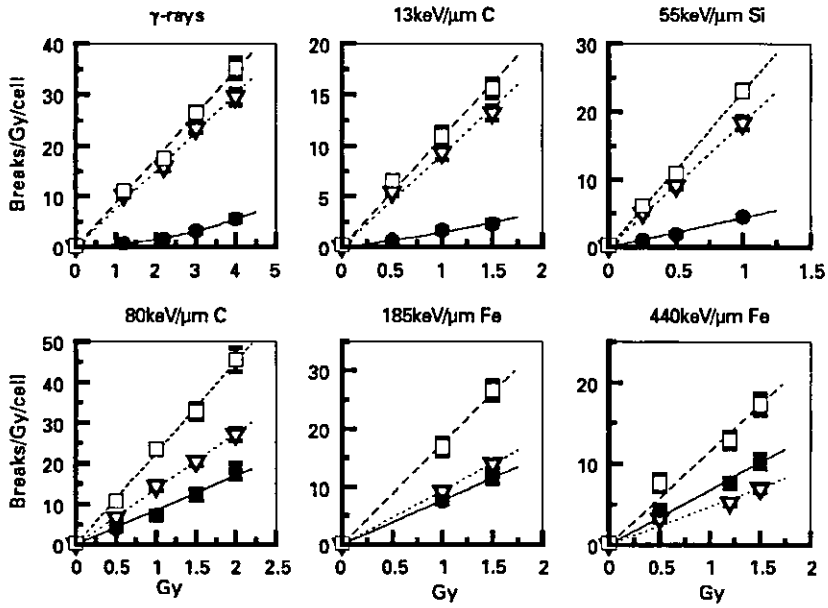

Fig. 2. Dose-response curves for the induction of chromatid-type breaks (▽), isochromatid-type breaks (●) and total chromatid breaks (□) after exposure to each type of radiation.

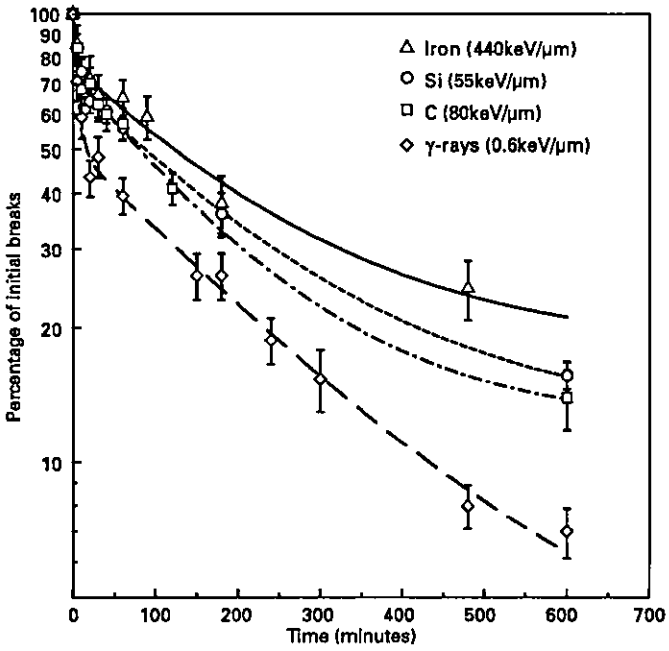

Fig. 3. Kinetics of rejoining of chromatid breaks following irradiation as a function of incubation time. Bars represent standard errors of the mean (data from Kawata et al., 2000).

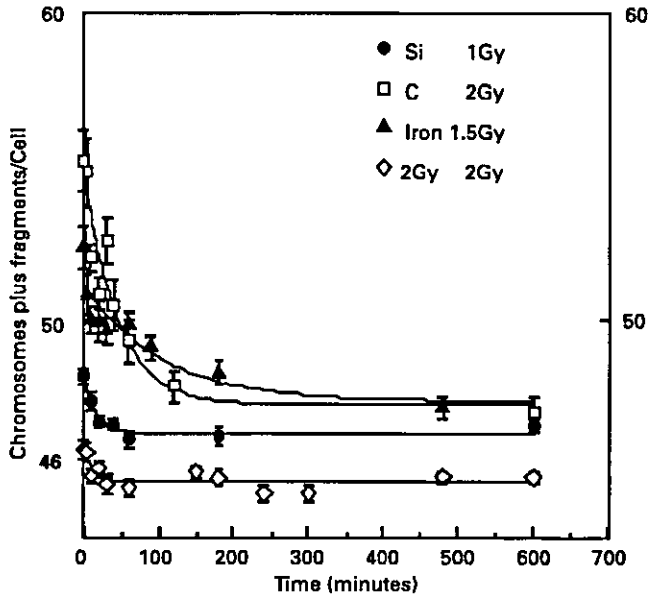

Fig. 4. Kinetics of rejoining of isochromatid breaks as a function of incubation times (Data from Kawata et al., 2001b).

Rejoining of chromatid breaks

The kinetics of total break (chromatid-type plus isochromatid-type), isochromatid break, and chromatid break rejoining were investigated after γ-rays, 13 keV/μm carbon, 55 keV/μm silicon, or 440 keV/μm iron particles (Kawata et al., 2000). The repair kinetics for total chromatid breaks showed a similar fast and slow time constant for both high-LET and γ-ray exposure (Fig. 3), and the half time for fast repair was about 4 min, regardless of radiation type. Iliakis and colleagues (Iliakis et al., 1993), using a combination of hypertonic treatment and fusion PCC technique, reported a half time of 1.5 min for repair of G1-phase CHO cells after exposure to X-rays. Durante and colleagues (Durante et al., 1998b), using the same technique with fluorescence in situ hybridization (FISH) analysis, also showed that γ-ray-induced chromosome breaks in G0 lymphocytes rejoined very quickly (half time of 5–6 min). Using the G2

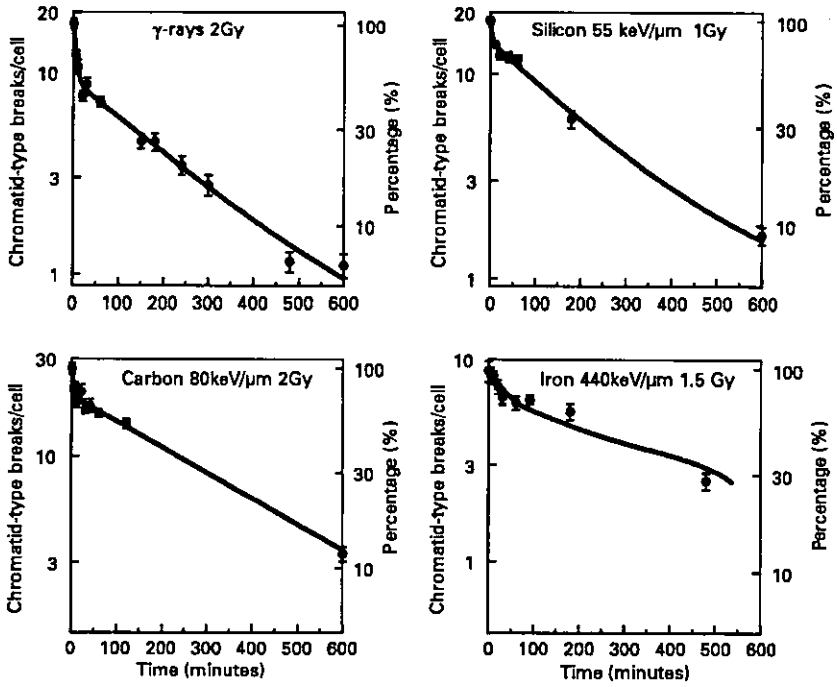

Fig. 5. Kinetics of rejoining of chromatid-type breaks as a function of incubation times (Data from Kawata et al., 2001b).

assay, Vral et al. (2002) demonstrated similar kinetics of disappearance of chromatid breaks following γ-rays and high-LET neutrons. These results suggest that the fast component of the repair process may be common throughout the cell cycle and independent of LET.

The percentage of residual breaks induced by high-LET exposure was from 4.2 to 6.2 times higher than γ-rays (Kawata et al., 2000), revealing an LET-dependent trend toward higher levels of residual chromatid breaks. Suzuki et al. (2001) also reported a higher frequency of residual chromatid breaks in human epithelial cells following high-LET iron particle exposure. Goodwin et al. (1994) demonstrated a clear LET-dependent trend in the percentage of excess residual fragments in CHO cells after helium (0.56 keV/μm), carbon (13.7 keV/μm), argon (115 keV/μm), and neon (183 keV/μm) particle exposure, with the reported percentage of residual excess fragments being 49% after 183 keV/μm neon particle exposure, compared to 11% after X-ray exposure. The higher rate of residual breaks induced by high-LET radiation may be due to the more clustered DNA damages induced by this type of exposure.

Kawata et al. (2001b) used calyculin-A-induced PCC method to examine the kinetics of isochromatid break rejoining, and found that high-LET radiation-induced isochromatid breaks rejoin quickly (Fig. 4). Since many more isochromatid breaks are produced by high-LET radiation, chromatid rejoining or exchange formation between isochromatid breaks is more likely to occur in these samples. During the isochromatid break rejoining or exchange formation process, a structural pattern similar to a simple chromatid-type break can be produced, which is therefore classified as residual chromatid-type break, leading to an increase in the number of chromatid-type breaks. This increase in chromatid breaks with repair time is not observed after low-LET radiation, since the initial yield of isochromatid breaks is much smaller. Therefore, the rejoining process of isochromatid breaks probably leads to the appearance of slower kinetics for chromatid-type break rejoining, especially for 440 keV/μm iron particles (Fig. 5).

Conclusion

High-LET radiation was found to be more effective at producing isochromatid breaks in the G2 phase of the cell cycle, and the repair process involved in the rejoining of these isochromatid breaks could explain why chromatid break yields remain higher after high-LET irradiation when compared with low-LET irradiation. The PCC technique with calyculin A proved very useful for analysis of the repair kinetics in G2 cells following low- or high-LET irradiation.

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Dominant human papillomavirus 16 infection in cervical neoplasia in young Japanese women; study of 881 outpatients

Nobuo Masumoto,[a] Takuma Fujii,[a,*] Mitsuya Ishikawa,[a] Makio Mukai,[b] Akiko Ono,[a] Takashi Iwata,[a] Kaneyuki Kubushiro,[a] and Shiro Nozawa[a]

[a] *Department of Obstetrics and Gynecology, Keio University School of Medicine, Shinjuku, Tokyo, 160-8582, Japan*
[b] *Department of Pathology, Keio University School of Medicine, Shinjuku, Tokyo, 160-8582, Japan*

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Abstract

Objective. Human papillomavirus (HPV) infection is reported to be related to carcinogenesis in the uterine cervix. In Japan, screening for cervical cancer by cytology is performed in women over 30 years old. The purpose of this study was to determine whether there is an association between patient age and cervical neoplasia or HPV infection in Japanese women.

Methods. Specimens from 881 randomly selected patients who came to our clinic were tested for HPV DNA by using Hybrid Capture II, whereas specimens from a 204-patient randomly selected subset diagnosed with cervical neoplasia were tested for HPV DNA by using polymerase chain reaction (PCR). HPV typing was performed in all the PCR-positive cases.

Results. The HPV-positive rate in the 20- to 29-year-old patients (29.0% in the normal cytology/histology group and 85.5% in the abnormal group) was higher than in the 30- to 59-year-old patients, and the rate declined until age 60 when age increased. While HPV 18, HPV 52, other HPV types, and HPV types as a whole were frequently detected in 30- to 49-year-old patients, HPV 16 was detected more frequently in the younger group than the other HPV types ($P = 0.03$). Among the HPV 16-positive patients with cervical neoplasia, the proportion of cervical intraepithelial neoplasia (CIN) 3 cases was high (44%) in the 20- to 29-year-old group.

Conclusions. Screening for cervical neoplasia by cytology should also be performed in women under 30 years old in Japan. The HPV typing could be a tool to strictly follow-up younger women who were diagnosed with CIN.

Keywords: Human papillomavirus 16; Cervical neoplasia; HPV typing

Introduction

Genital infection with human papillomavirus (HPV) is one of the most common sexually transmitted diseases, and its prevalence in young women ranges from 20% to 46% in various countries [1–5]. Genital HPV, for example, HPV 16, has been reported to be related to cervical cancer [6,7]. HPV 16 and HPV 18 have been reported to be the only two types included in the top quartiles for both incidence and duration of infection among approximately 20-year-old women in the United States [8]. HPV detection in other countries has also been reported to be high among young women and low among women around 30–50 years old [9,10]. In Japan, infection with HPV 16, 18, 31, 51, 52, and, 58 has been considered to be associated with a high risk of cervical cancer [11–13]; however, to our knowledge, there have been no large studies of an association between patient age and HPV infection. Screening for cervical lesions by cytology is performed in women over 30 years old in Japan; however, the number of young patients with cervical cancer has recently been increasing. One of the purposes of this study was to elucidate the incidence of HPV infection among young Japanese women. We performed the Hybrid Capture II (HCII) (Digene Inc., Silver Spring, USA) test and HPV typing of fluid-based specimens obtained from outpatients.

* Corresponding author. Department of Obstetrics and Gynecology, Keio University School of Medicine, 35 Shinanomachi, Shinjuku, Tokyo, 160-8582, Japan. Fax: +81-33226-1667.
E-mail address: fujiit@sc.itc.keio.ac.jp (T. Fujii).

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We previously reported a method for HPV-DNA transcript detection in cytology specimens by reverse transcriptase nested polymerase chain reactions (PCR) [14] and a method for detecting multiplex HPV infection by PCR single-stranded DNA-conformational polymorphism analysis [15]. We applied these methods to the fluid-based specimens and then directly sequenced the PCR products. We also conducted a prospective study on CIN cases infected with HPV to determine whether progression of CIN is different among HPV types. This study was undertaken to investigate epidemiology of HPV infection, especially to determine whether there is an association between patient age and cervical neoplasia or HPV infection in Japanese women.

Materials and methods

Specimens from 881 randomly selected patients who came to our clinic were tested for HPV DNA by using HCII, whereas specimens from a 204-patient randomly selected subset diagnosed with cervical neoplasia were tested for HPV DNA by using PCR. HPV typing was performed in all the PCR-positive cases.

Sample preparation

A total of 414 of the 3408 patients with normal cytology or histology and a total of 467 out of the 564 patients with abnormal cytology or histology who came to our clinic between October 2000 and April 2001 were randomized, and these 881 patients were enrolled in the study. The patient population consisted of a mixture of asymptomatic women and women who were being followed-up because of previous atypical smears or after the treatment of previous genital malignancies. Colposcopy and biopsy studies were performed in 535 of the 881 cases. ThinPrep sample vials with exfoliated cells were collected with a broom device (Cervex Brush; Unimar, Wilton, CT), stored at ambient temperature, and used for HPV-DNA analysis within 12 months of collection. Specimens from all 881 patients were tested for the presence of HPV DNA by using HCII, whereas specimens from 204 random cases diagnosed with having cervical neoplasia on the basis of biopsy studies were tested for HPV DNA by PCR. HPV typing and sequencing analysis were performed in all the PCR-positive cases. Informed consent was obtained from each participant in the biopsy study after an oral explanation of the study.

HPV detection using HCII

HPV DNA detection was performed using the commercially available HCII hybrid capture technique. Fluid-based specimens were analyzed for the presence of high-risk HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68

Table 1
Frequency of HPV infection in each category of patient's age

		Age (years)								
		10–19	20–29	30–39	40–49	50–59	60–69	70–79	80–89	90–	Total
Patients with normal cytology and histology	HPV (+)	0 (–)	9 (29.0%)	15 (13.0%)	15 (12.4%)	16 (15.2%)	10 (31.3%)	5 (55.6%)	0 (0%)	0 (–)	70 (16.9%)
	HPV (–)	0 (–)	22 (71.0%)	100 (87.0%)	106 (87.6%)	89 (84.8%)	22 (68.7%)	4 (44.4%)	1 (100%)	0 (–)	344 (83.1%)
Patients with abnormal cytology and/or histology	HPV (+)	1 (100%)	47 (85.5%)	142 (80.2%)	77 (69.4%)	44 (63.8%)	28 (71.8%)	11 (84.6%)	1 (100%)	1 (100%)	352 (75.4%)
	HPV (–)	0 (0%)	8 (14.5%)	35 (19.8%)	34 (30.6%)	25 (36.2%)	11 (28.2%)	2 (15.4%)	0 (0%)	0 (0%)	115 (24.6%)

using a Probe B cocktail. The enzyme-linked immunosorbent assay was based on a sandwich hybridization followed by a nonradioactive alkaline phosphatase reaction with chemiluminescence on microplates.

DNA extraction

Approximately 10 ml of preserved fluid was centrifuged at 3000 rpm for 30 min. The pellet was washed once in phosphate-buffered saline, followed by genomic DNA extraction using proteinase K and phenol–chloroform treatment. The quality and quantity of the extracted genomic DNA was monitored using ethidium bromide-stained agarose gel electrophoresis [12].

PCR

The presence of intracellular HPV DNA was determined using PCR analysis with consensus primer pairs (L1C1, L1C2) [16], designed to amplify an approximately 250-bp segment of DNA. These consensus primer pairs target the HPV L1 open reading frame and detect a broad range of genital HPVs. A 50-μl volume containing 20 mM Tris HCl buffer (pH 8.0), 50 mM KCl, 0.2 mM dNTP mix, 2 mM $MgCl_2$, 0.5 μM of each forward and reverse primer, 0.25 units of *Taq* polymerase (TaKaRa, Otsu, Japan), and 500 ng to 1 μg of template DNA was used for each reaction. After an initial period of denaturation at 95°C for 10 min, 43 cycles of reactions were performed, each consisting of denaturation at 95°C for 1.5 min, annealing at 48°C for 1.5 min, and extension at 70°C for 2 min. PK114/K, a variant HPV 16 clone provided by Dr. Mattias Durst, was used as a positive control.

Direct sequencing of PCR products

A partial L1 sequence was amplified by the primer L1C1. The amplified PCR products were purified, and automated sequencing was performed using an ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA). The HPV type was determined based on an approximately 200 bases of L1 sequence and a search of the NCBI database (GenBank sequences; http://www.ncbi.nlm.nih.gov/blast/Blast.cgi) using Sequencing Analysis 3.3 software (Perkin-Elmer Corporation, Norwalk, CT).

Statistics

Correlations between the rate of HPV infection in patients with normal cytology or histology and patients with abnormal cytology and/or histology and correlations between the frequency of cases with persistent cervical intraepithelia neoplasia (CIN) among the HPV 16-positive cases, HPV 52 cases, and all cases were analyzed by using the chi-square test. Correlations between the distribution of HPV 16-positive cases and the other HPV-positive cases in each age group were analyzed by using the Mann–Whitney's test. P values of 0.05 or less were considered to be statistically significant.

Results

The percentage of patients who were HPV-positive according to the results of the HCII was higher among those with abnormal cytology and/or histology (75.4%, 352 out of 467 cases) than among those with normal cytology and histology (16.9%, 70 out of 414 cases, $P < 0.0001$). The HPV-positive rate according to the HCII was higher in the 20- to 29-year-old group (29.0% in the normal cytology/histology group and 85.5% in the abnormal group) than in the 30- to 59-year-old group, and it tended to decline with age in these age groups. Although the number of patients over 60 years old was small, the HPV-positive rate tended to rise with age in this age group (Table 1).

Table 2 summarizes the HPV types in each age category. HPV 16, HPV18, HPV 51, HPV 52, other HPV types, and all HPV types as a whole were more frequently detected in the 30- to 49-year-old group. HPV 16 was the most common type in 20- to 49-year-old group, and it was frequently detected in younger age groups than the other HPV types ($P = 0.03$).

Table 3 shows the histological diagnoses according to age group. The proportion of CIN3 cases in the 20- to 29-year-old group among the HPV 16-positive patients with cervical lesions (44%) was higher than that among the other

Table 2
Summary of HPV type in each category of patient's age

Age (years)	HPV type																
	16	18	31	33	35	51	52	53	56	58	59	66	70	71	84	Others	Total
20–29	9		1			6	2						1			4	23
30–39	31	8	2		4	10	11		6	1	1	1		1	1	9	86
40–49	14	1		2	2	3	8	1		2						6	39
50–59	2	2	1			2	1		1		1				1	2	13
60–69		3				1	3				1					1	9
70–79							1									1	2
Total	56	14	4	2	6	22	26	1	7	3	3	1	1	1	2	23	172

Table 3
HPV type and histological study in each category of patient's age

		Age (years)						
		20–29	30–39	40–49	50–59	60–69	70–79	Total
HPV16	CIN1	1 (11.1%)	7 (25.9%)	2 (12.5%)	0 (0%)	0 (0%)	0 (–)	10
	CIN2	4 (44.4%)	6 (22.2%)	3 (18.8%)	0 (0%)	0 (0%)	0 (–)	13
	CIN3	4 (44.4%)	14 (51.9%)	7 (43.8%)	1 (33.3%)	0 (0%)	0 (–)	26
	SCC	0 (0%)	0 (0%)	4 (25.0%)	2 (66.7%)	1 (100%)	0 (–)	7
Others	CIN1	9 (56.3%)	22 (42.3%)	11 (45.8%)	5 (62.5%)	3 (37.5%)	1 (50%)	51
	CIN2	6 (37.5%)	16 (30.8%)	6 (25.0%)	0 (0%)	3 (37.5%)	0 (0%)	31
	CIN3	0 (0%)	12 (23.1%)	7 (29.2%)	1 (12.5%)	0 (0%)	0 (0%)	20
	SCC	1 (6.3%)	2 (3.8%)	0 (0%)	2 (25.0%)	2 (25.0%)	1 (50%)	8
Total		25	79	40	11	9	2	166

CIN = cervical intraepithelial neoplasia; SCC = squamous cell carcinoma.

HPV-positive patients (0%). The frequency of CIN3 cases among HPV 16-positive patients tended to increase with age until age 39, and that of SCC also tended to increase with age among over 40-year-old cases.

Table 4 shows the results of the prospective study of patients with CIN1 or CIN2 after biopsy was performed. Among the 113 CIN1 or CIN2 cases, the 19 lost to follow-up and the 23 cases in which vaporization was performed for CIN2 were excluded, and the remaining 71 cases, which were followed-up for more than 1 year, were studied. The follow-up period was 23 $\pm$ 4.7 months. The proportions of cases with persistent CIN for more than 1 year were 41.7% of the HPV 16-positive cases (5 out of 12 cases), 62.5% of the HPV 52-positive cases (5 out of 8 cases), and 45.0% of the total number of cases (32 out of 71 cases), and the differences between them were not significant. The proportion of 20- to 29-year-old patients with persistent CIN was 61.5% (8 out of 13 cases). The proportion tended to decline with age, and then rose to 75.0% (3 out of 4 cases) in the 60- to 69-year-old group (Table 5).

Discussion

Investigation of carcinogenesis in the uterine cervix has shown that HPV infection is a strong risk factor for cervical cancer [6,7]. The results of HCII in this study showed that 75.4% of patients with abnormal cytology and/or histology were HPV-positive, a much higher proportion than among those with normal cytology and histology (16.9%). The HPV-positive rate of Japanese women is similar to the rates in other countries, as previously reported [17]. The HPV-positive rate was strongly associated with age, being highest in the 20- to 29-year-old group and declining to the lowest in women around 35 years old, as found in other investigations [9,10]. This finding can be explained by acquired immunity to HPV from past exposure [18,19], and women who were immunosuppressed by infection with the human immunodeficiency virus are, in fact at high risk of HPV infection [20]. The HPV-positive rate then tended to rise in the over 60-year-old group in our study, and some studies have reported similar data for the relationship between age and detection of HPV among elderly women in Spain, Colombia, and Costa Rica [17,21]. The cause of this tendency is unclear, but atrophic mucosa in the postmenopausal cervix may be more intensely exposed to HPV and that may be why HPV infection occurs more easily than in younger age groups.

We also found that HPV 16 was more frequently detected in the younger age group than the other HPV types, and even in the 20- to 29-year-old group, CIN3 was detected in 44% of HPV 16-positive patients with cervical lesions. These results suggest that HPV 16 infection is a strong risk factor for CIN3 even in the young age group under 30. The US Food and Drug Administration has approved an HPV test and allowed it to be used in conjunction with the Pap test to screen for HPV infection in women over age 30 [22], and our results suggest that women under 30 years old should be tested for HPV infection if CIN is detected.

The peak incidence of cervical cancer in women has been reported to be in the over 40 age group [23,24]. We found

Table 4
Prospective study after biopsy in each HPV type

	HPV type												
	16	31	33	35	51	52	56	59	70	84	Others	Negative	Total
Persistent over 1 year	5	0	1	1	6	5	1	1	0	1	6	5	32
Disappeared after 1 year	7	1	0	1	6	3	2	1	1	0	8	9	39

Table 5
Prospective study after biopsy in each category of patient's age

	Age (years)				
	20–29	30–39	40–49	50–59	60–69
Persistent over 1 year	8 (61.5%)	14 (45.2%)	6 (35.3%)	1 (16.7%)	3 (75.0%)
Disappeared after 1 year	5 (38.5%)	17 (54.8%)	11 (64.7%)	5 (83.3%)	1 (25.0%)

that the frequency of CIN3 cases among HPV 16-positive patients tended to increase with age until age 39, and that of SCC also tended to increase with age among over 40-year-old cases. The finding is probably attributable to persistent infection with high-risk types of HPV, which is associated with carcinogenesis in the uterine cervix. Ho et al. [8] have reported that the longer an infection persists, the more difficult it is to recover, and thus patients with persistent HPV 16 infection should be followed-up strictly long-term.

Several studies on the prevalence of HPV infection have concluded that HPV infections are usually transient [25–29]. Other studies have reported that infection with high-risk types of HPV is a risk factor for persistent infection over 6 months in young women [8] and that older age is a risk factor for persistent HPV infection [30,31]. Our results showed that the proportion of patients with persistent CIN for more than a 1-year period was high in young age group. Therefore, we think that even young women with CIN must be strictly followed-up.

In conclusion, because HPV 16 infection and persistent CIN for more than a 1-year period were more frequently detected in the younger age group, we concluded that screening for cervical neoplasia by cytology should also be performed in women under 30 years old in Japan, and even young women should be carefully followed-up once CIN is diagnosed. The HPV typing for young women who were diagnosed with CIN can be a tool for the extraction of high-risk group for follow-up.

Special note

According to a notification of the ministry of Health, Labor, and Welfare in Japan, cervical cancer screening program will be revised in spring 2004 and the screening will be performed over for 20-year-old women every 2 years in Japan.

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Conservative excisional laser conization for early invasive cervical cancer

Masatsugu Ueda*, Ken Ueki, Masanori Kanemura, Shinji Izuma, Hiroyuki Yamaguchi, Yoshito Terai, Minoru Ueki

Department of Obstetrics and Gynecology, Osaka Medical College, Takatsuki, Osaka 569-8686, Japan

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Abstract

Objective. To investigate the possibility of conservative excisional laser conization for early invasive cervical cancer.

Methods. Four hundred one women with early invasive squamous cell cancer were treated by laser conization and semiradical or radical hysterectomy with pelvic lymphadenectomy. Their histologic findings and clinical outcomes were evaluated retrospectively.

Results. Two hundred Ia1 cases without confluent invasion or vessel permeation receiving only laser therapy had no recurrent disease. There was no lymph node metastasis in 123 Ia1 and 24 Ia2 cases with stromal invasion of under 4 mm in depth regardless of confluent invasion and vessel permeation. However, lymph node metastasis was detected in 1 of 13 Ia2 cases with stromal invasion of over 4 mm in depth and in 5 of 41 Ib1 cases. All of these six cases had vessel permeation in the resected specimens.

Conclusion. Conservative excisional laser conization may be possible for stage Ia cervical cancer with stromal invasion of under 4 mm in depth. However, the risk of lymph node metastasis should be still considered for those lesions with vessel permeation.

Keywords: Laser conization; Conservative management; Cervical cancer

Introduction

Cervical cancer is the second most common cancer in women worldwide, and is both a preventable and a curable disease especially if identified at an early stage. A recent analysis of five long-term studies of the follow-up of conservative treatment for cervical intraepithelial neoplasia (CIN) has shown a reduction in the risk of invasive cervical cancer by 95% for at least 8 years [1]. Conization of the cervix is widely used for the diagnosis and conservative treatment of CIN. Recently, the traditional surgical technique of cold knife conization has been replaced by laser conization and by the loop electrosurgical excisional procedure because of the high incidence of incomplete excision and recurrence with conventional cold conization [2]. The main advantage of these methods over the destructive procedures, such as cryosurgery and laser vaporization, is that they provide histologic information on the depth of invasion and the involvement of the surgical margins. We have performed neodymium-ytlium, argon, gadolinium (Nd-YAG) laser conization for over 2500 cases with cervical neoplasms so far and reported its usefulness as a conservative therapeutic tool for CIN and microinvasive cancer without vessel permeation and bulky invasion [3–6]. However, the number of young patients with more advanced disease has been increasing, and the necessity of conservative therapy for those lesions is now becoming greater to preserve their fertility. In the present study, we sought to find out the clinical and pathological limitation of conservative treatment for early invasive cervical cancer using laser technique.

Patients and methods

In the past 15 years, between 1983 and 1997, we treated 401 women with early invasive squamous cell

* Corresponding author. Department of Obstetrics and Gynecology, Osaka Medical College, 2-7 Daigakumachi, Takatsuki, Osaka 569-8686, Japan. Fax: +81 72 681 3723.

E-mail address: gyn017@poh.osaka-med.ac.jp (M. Ueda).

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cancer of the cervix. Their histologic findings and clinical outcomes were evaluated retrospectively. Nd-YAG laser conization was initially performed for 241 cases who were preoperatively suspected as having microinvasive squamous cell cancer by cytology, colposcopy, and target biopsy. A large dome-like contact laser conization was done and contact vaporization on the surrounding tissue and the ectocervix was added after the conization as described previously [3,4]. A histological examination was done on 16 blocks of each cone specimen stained with hematoxylin–eosin. Stages of the disease were classified according to FIGO classification [7] based on the histologic finding of the cone specimen; stage Ia1, measured invasion of stroma no greater than 3 mm in depth and no wider than 7 mm in diameter; Ia2, measured invasion of stroma greater than 3 mm but no greater than 5 mm in depth and no wider than 7 mm in diameter; Ib1, preclinical lesions greater than stage Ia.

Two hundred of 241 cases who had stage Ia1 disease without confluent invasion (confluent pattern of stromal growth) or vessel permeation (lymph vascular space invasion) received no additional surgical treatment because we previously demonstrated that no lymph node metastasis was observed in those lesions [4]. Forty-one (17%) of 241 cases underwent semiradical or radical hysterectomy and pelvic lymphadenectomy because they had stage Ia1 with confluent invasion or vessel permeation, Ia2 or Ib1 disease on the initial conization. The other 160 patients who were preoperatively suspected as having Ia2 or Ib1 disease by cytology, colposcopy, and target biopsy received radical surgery. Histological specimens of 401 patients enrolled in this study were re-reviewed by pathologists after surgery and were diagnosed as having squamous cell cancers in stage Ia1–Ib1. The depth of stromal invasion in resected specimens was compared with the diameter of stromal invasion and the incidence of confluent invasion, vessel permeation, or lymph node metastasis, and checked by the Mann–Whitney U and chi-square tests. A level of $P < 0.05$ was accepted as statistically significant.

Postoperatively, all patients were followed up every 3 to 6 months in our outpatient clinic with cytology, colposcopy, and/or biopsy until December 2003. The median follow-up time was 117.1 months with a range of 72–240 months.

Results

The operative procedure of laser conization required 12 min on the average. A blood loss of over 30 ml during the operation occurred in 11% and cervical obstruction occurred in 8% during the follow-up period.

Table 1 shows the correlation between depth and diameter of stromal invasion in 401 cases examined. Two hundred (62%) of 323 Ia1 cases without confluent invasion or vessel permeation were treated only by laser conization as described above. One hundred twenty-three (38%) of 323 Ia1 cases underwent abdominal surgery because they were preoperatively suspected as having stage Ia1 with confluent invasion or vessel permeation, Ia2 or Ib1 disease. Thirty-seven Ia2 and 41 Ib1 cases also underwent radical surgery. Increasing depth of stromal invasion was well correlated with increasing diameter.

Table 1
The correlation between depth and diameter of stromal invasion in 401 cases examined

Diameter of stromal invasion	Depth of stromal invasion			
	~3.0 mm (337)	3.1–4.0 mm (30)	4.1–5.0 mm (20)	5.1 mm~ (14)
Under 7 mm	Ia1 (323)	Ia2 (24)	Ia2 (13)	Ib1 (3)
Over 7 mm	Ib1[a,b,c] (14)	Ib1[a] (6)	Ib1[b] (7)	Ib1[c] (11)

Two hundred of 323 Ia1 cases without confluent invasion or vessel permeation were treated only by laser conization 123 of 323 Ia1, 37 Ia2, and 41 Ib1 cases underwent semiradical or radical hysterectomy and pelvic lymphadenectomy. ( ): number of cases.
[a] $P = 0.0002$.
[b,c] $P = 0.0001$.

Table 2 indicates the correlation between depth of stromal invasion and incidence of confluent invasion, vessel permeation or lymph node metastasis. Increasing depth of stromal invasion and stages were correlated with increasing incidence of confluent invasion and vessel permeation. In 323 Ia1 cases, the incidence of confluent invasion and vessel permeation was 3.7% (12/323) and 3.1% (10/323), respectively. Two hundred of 323 cases were treated only by laser conization as described above. Lymph node metastasis was not observed in 123 of 323 Ia1 cases who underwent semiradical or radical hysterectomy and pelvic lymphadenectomy. In 24 Ia2 cases with stromal invasion of under 4 mm in depth, the incidence of confluent invasion and vessel permeation was 16.7% (4/24) and 12.5% (3/24), respectively. However, there was no lymph node metastasis in these 24 cases. In contrast, lymph node metastasis was detected in 1 of 13 Ia2 cases with stromal invasion of over 4 mm in depth and in 5 of 41 Ib1 cases. All of these six cases had vessel permeation in the resected specimens.

Of 200 Ia1 cases without confluent invasion or vessel permeation receiving only laser therapy, 11 cases had positive cone margins with CIN I to III. Two cases with CIN III received re-conization and one with CIN III underwent re-vaporization. The other eight cases with CIN I to II experienced spontaneous disappearance of their lesions during follow-up period, which ranged from 9 to 47 months after initial laser conization. All of 200 patients treated only by laser therapy had no recurrent disease. Final pathology results of 41 cases who initially had laser conization followed by hysterectomy were 3 Ia1, 10 Ia2 and 28 Ib1 diseases. One Ia2 and five Ib1 cases with pelvic lymph node metastasis subsequently received an additional radiation therapy and had no recurrent disease. After all,

Table 2
The correlation between depth of stromal invasion and incidence of confluent invasion, vessel permeation or lymph node metastasis in 401 cases examined

Variable	Ia1	Ia2		Ib1			
	−3.0 mm	3.1–4.0 mm	4.1–5.0 mm	−3.0 mm	3.1–4.0 mm	4.1–5.0 mm	5.1 mm⁻
Confluent invasion	12/323[a,b]	4/24 10/37[a]	6/13	2/14 15/41[b]	3/6	3/7	7/14
Vessel permeation	10/323[c,d]	3/24 8/37[c]	5/13	2/14 16/41[d]	4/6	3/7	7/14
Lymph node metastasis	0/123	0/24 1/37[e]	1/13	1/14 5/41[e]	0/6	1/7	3/14

Two hundred of 323 Ia1 cases without confluent invasion or vessel permeation were treated only by laser conization. Lymph node metastasis was not observed in 123 of 323 Ia1 cases who underwent semiradical or radical hysterectomy and pelvic lymphadenectomy.
[a–d] $P < 0.0001$.
[e] Not significant.

none of the 401 patients enrolled in this study have recurred so far during follow-up period.

Discussion

We previously suggested that laser conization might be an acceptable conservative therapy for stage Ia1 and selected Ia2 cases without confluent invasion or vessel permeation based on the clinical analysis of 227 patients with early invasive squamous cell cancer of the cervix [6]. Our present results on 401 patients preoperatively suspected as having early invasive cancer demonstrated that there was no lymph node metastasis in 123 Ia1 and 24 Ia2 cancer with stromal invasion of under 4 mm in depth regardless of confluent invasion and vessel permeation. Moreover, none of the 401 patients enrolled in this study including 200 Ia1 cases treated only by laser conization had no recurrent disease during the long follow-up period which ranged from 72 to 240 months with a median time of 117.1 months. Creasman et al. [8,9] reported that conservative therapy was possible for stage Ia1 and some stage Ia2 patients, and Sevin et al. [10] advised that conization for stage Ia patients might be possible but should be performed based not only on depth of invasion but also on vessel permeation. Recently, Elliott et al. [11] demonstrated that stage Ia1 patients could be managed only by conization. However, the clinical and pathological criteria for conservative treatment of stage Ia2 squamous cell cancer of the cervix has not been established yet.

In the conservative therapy for Ia2 cancer, it is quite important to determine the risk of lymph node metastasis. The incidence of pelvic lymph node metastasis in stage Ia2 disease has been reported to be 0/44 (0%) [9], 2/59 (3.4%) [11], 2/28 (7.1%) [12], 7/94 (7.4%) [13], and 2/9 (28.6%) [14]. Lymph vascular space invasion was found in 11/44 (25%) [9], 30/59 (53%) [11], 15/28 (54%) [12], 31/94 (33%) [13], and 7/9 (77.8%) [14], respectively. These previous reports indicated that lymph node metastasis was closely associated with lymph vascular space invasion in resected cervical lesions. In our series, 1 Ia2 and 5 Ib1 patients with pelvic lymph node metastasis also had vessel permeation in the resected specimens. In contrast, the rate of lymph node metastasis in stage Ia1 disease was reported to be 1/679 (0.15%) from a number of literatures [4]. The risk of lymph node metastasis with vessel permeation in Ia2 cancer may be significantly higher than that in Ia1 cancer. NIH consensus statement [15] also suggested that radical surgery with lymphadenectomy is needed for Ia2 cancer because of the high incidence of pelvic lymph node metastasis. In order to preserve the fertility of the patient with stage Ia2 disease on initial conization, laparoscopic lymph node sampling or dissection may be recommended in the present stage [16].

Despite abundant evidences on the correlation between lymph node metastasis and vessel permeation in stage Ia2 cervical cancer, the limit of stromal invasion for conservative excisional laser conization in Ia2 cancer has not been well discussed. Only Zaino et al. [17] reported that lymph node metastasis was strongly associated with the depth of invasion and no lymph node metastasis was found in the cases with stromal invasion of under 4 mm in depth. The present results that no lymph node metastasis was found in stage Ia cervical cancer with stromal invasion of under 4 mm in depth may suggest the possibility of conservative laser therapy for those lesions regardless of confluent invasion and vessel permeation. Although the risk of lymph node metastasis should be still considered for Ia2 cancer with vessel permeation according to the previous literatures, our observations may be helpful for active challenge to conservative management of the patients with early invasive cervical cancer in reproductive age.

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