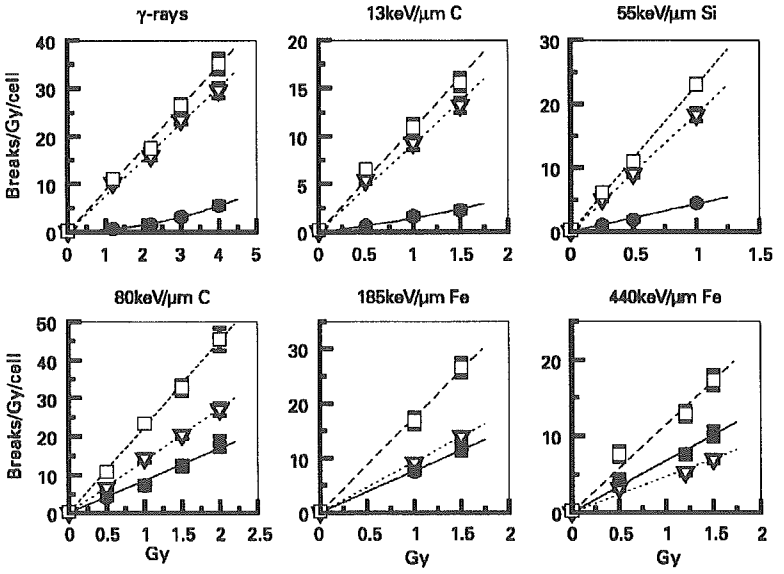

Fig. 2. Dose-response curves for the induction of chromatid-type breaks (▽), isochromatid-type breaks (●) and total chromatid breaks (□) after exposure to each type of radiation.

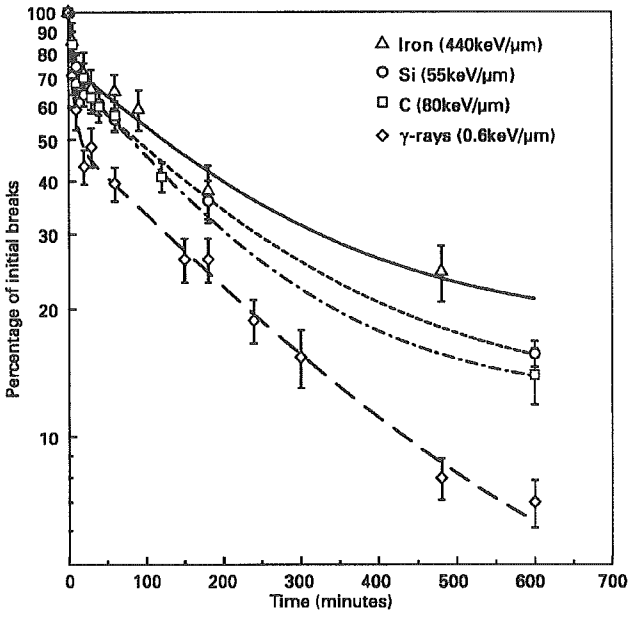

Fig. 3. Kinetics of rejoining of chromatid breaks following irradiation as a function of incubation time. Bars represent standard errors of the mean (data from Kawata et al., 2000).

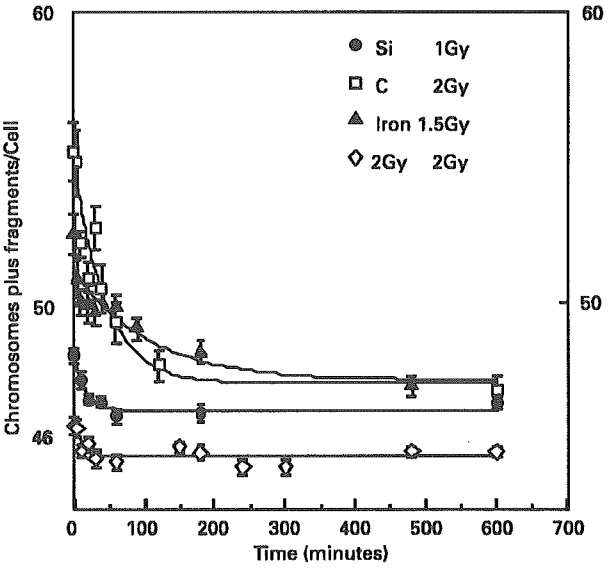

Fig. 4. Kinetics of rejoining of isochromatid breaks as a function of incubation times (Data from Kawata et al., 2001b).

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

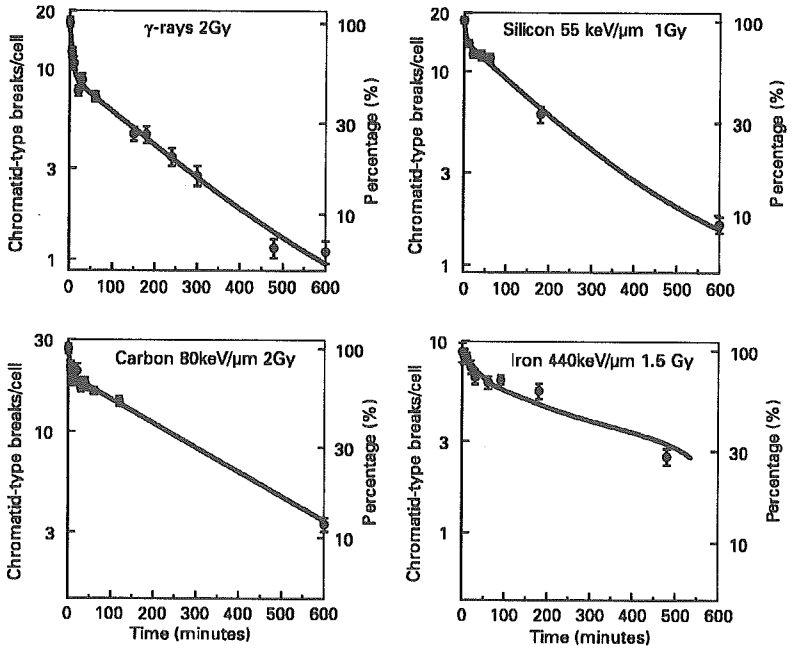

Fig. 5. Kinetics of rejoining of chromatid-type breaks as a function of incubation times (Data from Kawata et al., 2001b).

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 ± 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[c]	1/13	1/14 5/41[c]	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] $P < 0.0001$.
[c] 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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Phase II study of irinotecan combined with mitomycin-C for advanced or recurrent squamous cell carcinoma of the uterine cervix: the JGOG study

Naohiko Umesaki[a,*], Tuneo Fujii[b], Ryuichiro Nishimura[c], Tetsuji Tanaka[a], Masato Nishida[d], Hiroshi Fushiki[e], Ken Takizawa[f], Kaichiro Yamamoto[g], Kazuo Hasegawa[h], Rikuichi Izumi[i]
for the Japan Gynecologic Oncology Group

[a]*Department of Obstetrics and Gynecology, Wakayama Medical University, Wakayama, Japan*
[b]*Department of Obstetrics and Gynecology, National Kure Hospital, Kure, Japan*
[c]*Department of Gynecology, Hyogo Medical Center for Adults, Akashi, Japan*
[d]*Department of Obstetrics and Gynecology, National Kasumigaura Hospital, Ibaragi, Japan*
[e]*Department of Obstetrics and Gynecology, Tonami General Hospital, Tonami, Japan*
[f]*Department of Obstetrics and Gynecology, Mitsui Memorial Hospital, Tokyo, Japan*
[g]*Department of Obstetrics and Gynecology, Kinki University Medical School, Sakai, Japan*
[h]*Department of Obstetrics and Gynecology, Kakogawa Hospital, Kakogawa, Japan*
[i]*Department of Obstetrics and Gynecology, Toyamma Medical and Pharmaceutical University, Toyama, Japan*

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Abstract

Objectives. The efficacy and toxicity of combined therapy with irinotecan (CPT-11) plus mitomycin-C (MMC) were evaluated in patients with advanced or recurrent squamous cell carcinoma (SCC) of the uterine cervix.

Methods. CPT-11 (100 mg/m^2) was administered on days 1, 8, and 15 by intravenous (iv) infusion over 90 min, while MMC (10 mg/m^2 iv) was given on day 1. This regimen was repeated every 28 days and at least two courses were given.

Results. Among 51 eligible patients (median age: 52 years; range: 25–72 years), 2 showed complete response (CR) and 24 showed PR, for an overall response rate (ORR) of 51.0% (95% confidence interval: 36.6–65.3%). In patients without prior chemotherapy, the ORR was 54.8% (38.7–70.2%). Twenty-five patients (Ib2:3, IIb:17, and IIIb:5) received this regimen as neoadjuvant chemotherapy and their ORR was 76% (54.9–90.6%). Twenty-two patients were able to undergo radical surgery after NAC. The major toxicity was neutropenia, which was grade 3–4 in 59% of the patients. Grade 3–4 thrombocytopenia and anemia were also seen in 26% of the patients each. The most common nonhematologic toxicity was diarrhea (grade 3–4 in 12%).

Conclusion. CPT-11 combined with MMC can be effective against advanced or recurrent SCC of the uterine cervix.

Keywords: Chemotherapy; Cervical carcinoma; CPT-11; MMC

Introduction

After the introduction of screening using Papanicolaou smears, the incidence of invasive cervical cancer decreased and it now only holds third place among gynecologic malignancies. Although the mortality rate from cervical cancer has also been decreasing, the 5-year survival rate of

* Corresponding author. Department of Obstetrics and Gynecology, Wakayama Medical University, 811-1, Kimiidera, Wakayama 641-0012, Japan. Fax +81 73 441 0630.
E-mail address: umesaki@wakayama-med.ac.jp (N. Umesaki).

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patients with advanced or recurrent cancer has not improved worldwide over the last two decades [1], probably because treatment has not changed significantly.

Recent improvements in chemotherapy may lead to longer survival by combining chemotherapy agents with radiation or surgery.

After the effectiveness of cisplatin for cervical carcinoma was demonstrated, combination therapy based on cisplatin was introduced. Such regimens have achieved response rates of 16–67%, but the complete response (CR) rate is less than 20% [2–4]. Bleomycin (BLM) is often used in combination therapy and the BOMP [5] regimen or BIP [6] regimen is well known to be effective for cervical cancer. However, BLM often causes serious side effects such as pneumonitis. Therefore, a new regimen containing cisplatin without BLM would be desirable.

Irinotecan hydrochloride (CPT-11) has also demonstrated potent antitumor activity against cervical carcinoma [7]. Therefore, we tested combination chemotherapy with cisplatin plus CPT-11 and found that the response rate to this regimen was 59% for advanced or recurrent disease [8] and 78% when it was used as NAC [9].

Because advanced or recurrent cervical cancer is often complicated by ureteral stenosis or obstruction, it can be difficult to use cisplatin, suggesting that a new regimen without cisplatin should be developed. On the basis of in vitro and in vivo studies, mitomycin-C (MMC) was selected as a drug to use with CPT-11 [10].

MMC has already been used to treat cervical carcinoma [11,12], so the efficacy of CPT-11 combined with MMC can be expected. Improvement of the QOL was also predicted because the regimen would not cause symptoms such as nausea or vomiting related to cisplatin.

Accordingly, we conducted a prospective clinical trial to evaluate the therapeutic activity and toxicity of CPT-11 plus MMC as chemotherapy for advanced or recurrent cervical cancer.

Patients and methods

Patient selection

Patients had to fulfill the following eligibility criteria: histologically proven cervical cancer of stage Ib, IIb, III, or IV, or recurrent disease, as well as at least one measurable tumor documented radiographically. In all patients, primary radiotherapy and chemotherapy were completed more than 1 month earlier. Other eligibility criteria were as follows: age ≤75 years, performance status (WHO) ≤2, adequate bone marrow reserve (leucocyte count of 4.0–12.0 × 10^3/μl, platelet count ≥100 × 10^3/μl, and hemoglobin ≥9.0 g/dl), and adequate renal and hepatic function (serum creatinine ≤2 mg/dl, BUN ≤30 mg/dl, and AST/ALT ≤2× the upper limit of normal). All subjects gave written informed consent to the study.

Patients were excluded for any of the following reasons: metachronous or synchronous other cancer, concurrent infection; preexisting diarrhea, ileus, or bowel obstruction; interstitial pneumonia or pulmonary fibrosis; massive ascites; pleural effusion; uncontrolled diabetes; or a history of severe drug hypersensitivity.

Regimen

An intravenous (iv) infusion of CPT-11 (100 mg/m^2 over 90 min) was given on days 1, 8, and 15. After completion of CPT-11 infusion on day 1, MMC (10 mg/m^2) was administered as an intravenous bolus. Granulocyte colony-stimulating factor (G-CSF) was administered if grade 3 neutropenia occurred with fever ≥38.0°C or if grade 4 neutropenia developed with or without fever. This treatment schedule was repeated every 4 weeks for two or three cycles.

Doses and the treatment schedule were modified to avoid severe side effects. CPT-11 was not given on day 8 or 15 if the leucocyte count or platelet count was <3.0 × 10^3/μl or <100 × 10^3/μl, respectively. Treatment was also withheld if the patient developed diarrhea ≥grade 1 according to the Eastern Cooperative Oncology Group scale [13]. Before the next course was started, the leucocyte count had to be ≥4.0 × 10^3/μl and the platelet count ≥100 × 10^3/μl. In addition, there had to be no diarrhea, and both liver and renal function had to meet the initial eligibility criteria. Dose modification was not done for low blood cell counts or diarrhea during the same course. Additionally, if the leucocyte count was <1.0 × 10^3/μl, the platelet was <50 × 10^3/μl, or diarrhea was ≥grade 2 during any course, the dose of CPT-11 was reduced to 80 mg/m^2 for the next course.

This trial was approved by the review board of the Japanese Gynecologic Oncology Group and by the institutional review board of each participating hospital.

Evaluation of response

The criteria for assessment of tumor response were as follows: complete response (CR) was defined as the complete disappearance of all known disease for a minimum of 4 weeks; partial response (PR) was defined as a ≥50% reduction in the sum of the length × width product of all measurable lesions for a minimum of 4 weeks; progressive disease (PD) was defined as a ≥25% increase in the sum of the products of all measurable lesions, reappearance of any lesion that had disappeared, or appearance of any new lesions; and stable disease (SD) was any outcome that did not qualify as response or progression.

Patients were considered to be evaluable for toxicity if they received at least one full course of per protocol therapy. Toxicity was evaluated according to WHO criteria [14], except that diarrhea was assessed by the Eastern Cooperative Oncology scale [13].

Statistical methods

The response rate and its 95% confidence intervals (95% CI) were calculated using a binomial distribution [15].

Results

Between August 1997 and March 2002, 63 women entered this trial under the supervision of the Japanese Gynecologic Oncology Group. Ten patients were ineligible for the following reasons: four patients had a low WBC count, three patients had no measurable disease, two patients had an inadequate drug-free period, and one patient had an adrenal tumor. Among the 53 eligible patients, two patients were not evaluated for response because one patient refused to actually undergo treatment after enrollment and because of a protocol error in one patient.

Table 1 shows the characteristics of the eligible patients.

They received a median of two courses of therapy (range: 1–5 courses) and the median age was 52 years (range: 25–72 years). Sixteen patients had recurrent disease (31.4%) and 35 patients (66.7%) had advanced primary disease. Thirty-four patients (55.8%) had not received previous treatment, while 17 patients (35.3%) had already undergone treatment. Among these 17 patients, chemotherapy had been given to nine patients (17.6%) and radiotherapy had been performed in eight patients (15.7%).

Table 1
Characteristics of eligible patients

Characteristics	No. of patients	(%)
Overall	51	(100)
Age (years)		
Median	52	
Range	25–72	
Performance status		
0	42	(82.4)
1	6	(11.8)
2	3	(5.9)
Primary or recurrent		
Primary	35	(68.6)
Recurrent	16	(31.4)
Prior therapy		
No	34	(66.7)
Yes	17	(33.3)
Chemotherapy	9	(17.6)
Radiotherapy	8	(15.7)
Site of disease		
Pelvic	43	(84.3)
Cervical	34	(66.7)
Others	11	(21.6)
Metastatic site	13	(25.5)
Lymph nodes	8	(15.7)
Lung	5	(9.8)
Liver	2	(3.9)

Response to therapy

There were 2 CRs and 24 PRs, for an overall response rate (ORR) of 51.0% (95% CI: 36.6–65.3%). Eighteen patients showed SD and three had PD. The remaining four patients could not be evaluated. Table 2 shows the responses stratified according to various clinical characteristics. In patients with primary cancer, the overall response rate was 62.9% (95% CI: 44.9–78.5). In patients with recurrent disease, on the other hand, the overall response rate was only 25% (95% CI: 7.3–52.4). For patients without prior therapy (chemotherapy or radiation therapy), the overall response rate was 58.8% (95% CI: 40.7–75.4), while the overall response rate was only 35.3% (95% CI: 14.2–61.7) for patients with prior therapy. In patients without prior chemotherapy, overall responses rate was 54.8% (95% CI: 38.7–70.2). In patients with prior chemotherapy, overall responses rate was 33.3% (95% CI: 7.5–70.1).

When measurable sites were analyzed, the following response rates were observed: primary site, 23/43 cases (53.5%); lymph nodes, 4/8 cases (50%); lung 1/5 cases (20%), and liver, 1/2 cases (50%).

Twenty-five patients (stage Ib2, 3; IIb, 17; and IIIb, 5) received neoadjuvant chemotherapy with this regimen. Among the 25 patients, there was 1 CR and 18 PRs, for an overall response rate of 76% (95% CI: 54.9–90.6%). Six patients had SD and no PD was observed. Radical surgery was performed after NAC in 22 of the patients. One patient with stage IIb disease and PR after NAC received radiotherapy because consent for surgery was not obtained. One patient with stage IIIb disease and CR after NAC also underwent radiotherapy. Surgery was done for 22 of the other 23 patients.

Among the 51 patients, 23 died of cancer-related death and the median overall survival time was 21.7+ months (range: 3.4–68.4+ months). Among patients with recurrent and stage IVB disease, 18 died of cancer-related death, with a median overall survival time of 8.6 months (range: 3.4–28.2 months).

Toxicity

Table 3 lists the significant toxicities encountered during study. Leukopenia and neutropenia were the major dose-limiting toxicities. Grade 3 or worse anemia was noted in 13 patients (25.5%) and grade 3 or worse thrombocytopenia was also seen in 13 patients (25.5%). Twenty-nine patients (56.9%) developed at least grade 1 diarrhea during treatment and 16 patients (31.4%) had grade 2 or worse diarrhea. Grade 3 or 4 diarrhea occurred in six patients (11.8%). Grade 3 anorexia and alopecia were observed in five patients (9.8%) each, but grade 3 nausea and vomiting only occurred in two patients (3.9%). In the first course, 28 patients (54.9%)

Table 2
Response to the irinotecan/mitomycin C treatment

Overall	No. of patients	CR	PR	NC	PD	NE	Response rate (%)
	51	2	24	18	3	4	51.0
Performance status							
0	42	2	20	14	2	4	52.4
1	6		2	4			33.3
2	3		2		1		66.7
Primary or recurrent							
Primary	35	1	21	8	1	4	62.9
Stage Ib	4		3	1			75
Stage II	19		12	5		2	63.2
Stage III	8	1	5	1		1	75
Stage IV	4		1	1	1	1	25
Recurrent	16	1	3	10	2		25
Prior therapy							
No	34	1	19	9	1	4	58.8
Yes	17	1	5	9	2		35.3
Chemotherapy							
No	42	2	21	13	2	4	54.8
Yes	9		3	5	1		33.3
Radiotherapy							
No	36	1	20	10	1	4	58.3
Yes	15	1	4	8	2		33.3
Site of disease							
Primary site	43	1	22	14	2	4	53.5
Cervical	34	1	18	10	1	4	
Others	9		5	7	1	2	
Metastatic site	13	1	5	4	3		46.2

received the full scheduled dosage of CPT-11 (three doses per course), and CPT-11 was omitted in 19.6% on day 8 and in 39.2% on day 15. The main reason for omission of CPT-11 was leukopenia. As a result, the actual dose intensity of CPT-11 was 53.8 mg/m^2 per week versus the protocol dose intensity of 75.0 mg/m^2 per week.

There were no deaths attributable to toxicity.

Discussion

Irinotecan hydrochloride (CPT-11) is a derivative of camptothecin with potent antitumor activity. The antitumor effect of CPT-11 is related to the inhibition of DNA topoisomerase I, which is a novel mechanism different from those of other anticancer agents. CPT-11 shows strong activity against various experimental tumors and there is little cross-resistance with other antitumor agents. Clinical trials have shown that CPT-11 is active against various cancers, including cervical cancer.

We searched for an agent other than cisplatin to use in combination with CPT-11 [10]. We selected effective agents against cervical cancer by an in vitro assay using three epidermoid cell lines (keratinizing, large cell non-keratinizing, and small cell non-keratinizing types of cervical cancer). We also confirmed the effectiveness of the chemotherapy agents by a test using xenografted tumors in nude mice. These studies revealed that MMC plus cisplatin was the most effective combination followed by BLM plus cisplatin, CPT-11 plus cisplatin, and CPT-11 plus MMC. The most effective agent other than cisplatin for combination with CPT-11 was MMC. Kano et al. [16] reported that CPT-11 had a marginal supra-additive effect when combined with MMC, and they recommended the simultaneous administration of CPT-11 and MMC for clinical application in treating gynecologic malignancies. MMC inhibits the

Table 3
Toxicities of the irinotecan/mitomycin C treatment

Toxicity	No. of patients	Grade					Total	%	Grade 3 4	%
		0	1	2	3	4				
Hematologic										
Leukopenia	51	4	5	14	20	8	47	(92)	28	(55)
Neutropenia	51	13		8	19	11	38	(75)	30	(59)
Anaemia	51	8	6	24	13		43	(84)	13	(26)
Thrombocytopenia	51	27	6	5	7	6	24	(47)	13	(26)
Gastrointestinal										
Diarrhea	51	22	13	10	5	1	29	(57)	6	(12)
Nausea or vomiting	51	10	20	20	2		42	(82)	2	(4)
Anorexia	51	13	17	16	5		38	(75)	5	(10)
Others										
Alopecia	51	19	17	10	5		32	(63)	5	(10)
Hepatic function disorder	51	49	1	1			2	(4)		
AST (GOT)	51	50			1		1	(2)	1	(2)
AST (GPT)	51	50			1		1	(2)	1	(2)
ALP	51	50		1			1	(2)		
Paralysis intestinal	51	50	1				1	(2)		
Abdominal pain	51	50	1				1	(2)		
Infection	51	50	1				1	(2)		
Rash	51	49			2		2	(4)	2	(4)

cleavage of DNA, so synergism between CPT-11 and MMC may occur because alkylating agents could make some CPT-11-induced DNA damage irreparable.

Villalona-Calero and Kolesar [17] reported that MMC was a modulator of CPT-11 activity because it increased topoisomerase I expression.

MMC was reported to achieve a response rate of 22% for cervical cancer [18] and has been used to treat cervical cancer in combination with many agents. BM [12] and BOMP [5] were well-known chemotherapy regimens for cervical cancer. These facts suggested that MMC plus CPT-11 could be a useful new chemotherapy regimen.

The schedule and the dose of CPT-11 and MMC were based on previous reports. CPT-11 was administered on days 1, 8, and 15 according to the regimen for a phase II study [7]. MMC was administered on day 1 because this was the day of cisplatin administration in the combined CPT-11 and cisplatin regimen. MMC shows dose-dependent activity, so it was administered by bolus injection [19]. The doses of CPT-11 and MMC were determined according to other reports [20,21].

Previously, CPT-11 plus MMC has been used for ovarian carcinoma. Shimizu et al. [20] reported that CPT-11 was administered at a dose of 120 mg/m^2 intravenously (iv) on days 1 and 15, while MMC was given intravenously at a dose of 7 mg/m^2 on days 1 and 15. This regimen was found to be effective for platinum-refractory clear cell or mucinous cyst adenocarcinoma of the ovary and toxicity was acceptable (including manageable hematologic reactions, diarrhea, nausea or vomiting, and alopecia).

Takizawa et al. [21] demonstrated that 100 mg/m^2 of CPT-11 and 5 mg/m^2 of MMC at 2-week intervals were reasonably well tolerated, while Villalona-Calero and Kolesar [17] used MMC (6 mg/m^2 on day 1) plus CPT-11 (125 mg/m^2 on days 2 and 8) to treat breast or esophageal (cardiac) adenocarcinoma. Based on these reports, we selected three doses of CPT-11 (100 mg/m^2) at 1-week intervals plus MMC (10 mg/m^2 on day 1).

In our regimen, the dose intensity of CPT-11 was 75 mg/m^2 per week and that of MMC was 2.5 mg/m^2 per week. Although our regimen had a higher dose intensity compared with these other reports, the actual CPT-11 dose intensity delivered to the patients was 58 mg/m^2 per week. This dose intensity of CPT-11 was similar to that for the regimen of Shimizu et al.

The response rate was 51% for advanced or recurrent cervical cancer. Several combination chemotherapy regimens have been tested in phase II studies [6,22–24] and objective responses have been documented in 30–70% of patients. However, it is difficult to compare the results of these studies because of the relatively small number of subjects and biases of patient selection. We previously performed a phase II study of CPT-11 and cisplatin as first-line chemotherapy for advanced or recurrent cervical cancer [8]. The eligibility criteria and clinical characteristics of the patients were similar to those of this study, so we were able to compare the response to CPT-11 plus cisplatin with that to CPT-11 plus MMC. As a result, we found no difference between these two regimens and CPT-11 plus MMC showed moderate activity against cervical cancer.

In recent years, neoadjuvant chemotherapy has been extensively investigated in patients in patients with cervical cancer. Of the 51 patients entered in this study, 25 patients (49%) were registered as having neoadjuvant chemotherapy. The response rate was 76% (19/25), which is similar to previous reports [9].

The most frequent grade 3–4 toxicities were neutropenia and thrombocytopenia. The frequency of neutropenia was lower than with other regimens, such as CPT-11 + CDDP [8], CDDP + IFM [25], or CDDP + IFM + BLM [25]. On the other hand, thrombocytopenia was more frequent. One possible explanation for this finding is that the pattern of hematological toxicity differs between CPT-11 and MMC, with neutropenia being typical of the former and thrombocytopenia being typical of the latter [26]. G-CSF is effective for elevating the neutrophil count, but there is no treatment for thrombocytopenia except platelet transfusion. Therefore, thrombocytopenia is a problematic toxicity of this regimen. Fortunately, platelet transfusion was not needed in this study, but reduction of the MMC dose for the next course needs to be considered if grade 3–4 thrombocytopenia occurs. Diarrhea is the most important nonhematologic toxicity of CPT-11. The frequency of grade 3–4 diarrhea was reported to be 19.2% in a late phase II study of CPT-11 [7]. The same dose of CPT-11 was used in the present trial and MMC was added, but grade 3–4 diarrhea only occurred in 12%. A lower frequency of diarrhea was achieved in this study because many of the subjects were previously untreated and because we became more familiar with the toxicities of CPT-11.Although the frequency of diarrhea was reduced, it still caused impairment of QOL. Therefore, diarrhea needs to be managed carefully. Recently, hangeshashintou [27,28] and loperamide [29] were found to be useful for preventing diarrhea induced by CPT-11 therapy, so these medicines should be used more actively.

In summary, CPT-11 plus MMC showed moderate activity against cervical cancer. Furthermore, this regimen does not need hydration and nausea or vomiting is rare, so the QOL is also well.

In conclusion, CPT-11 plus MMC showed a useful regimen for advanced and recurrent cervical cancer.

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ORIGINAL PAPER

F. Kimura · J. Watanabe · H. Hata · T. Fujisawa
Y. Kamata · Y. Nishimura · T. Jobo · H. Kuramoto

PTEN immunohistochemical expression is suppressed in G1 endometrioid adenocarcinoma of the uterine corpus

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Abstract *Purpose*: PTEN is a tumor suppressor gene that inhibits cell proliferation by regulating intracellular signaling pathways, and this activity can be abolished by mutations of the PTEN gene. This study was designed to examine the correlation of PTEN expression with the expression of cell cycle regulators and with clinicopathological parameters in endometrioid adenocarcinoma of the uterine corpus. *Methods*: Tissue samples of 117 endometrioid adenocarcinomas in addition to those of 19 normal endometria and 20 endometrial hyperplasias were used for the study. Immunohistochemical staining for PTEN protein was performed with the labeled streptavidin-biotin method on formalin-fixed and paraffin-embedded tissue samples. PTEN expression was represented as the staining score. *Results*: Immunohistochemistry showed that the nuclei of cells were positive for PTEN. The PTEN staining score of normal endometrium was significantly higher in the proliferative phase than in the secretory phase. The scores of various endometrial hyperplasias were not significantly different from each other, regardless of the type of hyperplasia. The PTEN staining scores of endometrioid adenocarcinomas were 7.6 ± 5.2 in G1, 9.6 ± 5.2 in G2, and 11.9 ± 3.7 in G3, and increased significantly as the histological grade increased. PTEN staining score was not significantly correlated with clinicopathological parameters such as FIGO stage, myometrial invasion, lymph-vascular space invasion (LVSI), lymph node metastasis or group, but was significantly correlated with labeling indices (LIs) of cell cycle regulators such as Ki-67, cdk2, cyclin A, cyclin D1, cyclin E, p27, and p53. The PTEN staining score of p53-wild cases was significantly lower than that of p53-mutant ones, but there was no significant difference of the score in cases with different PTEN gene status. PTEN expression was significantly lower in cases with both high levels of estrogen receptor and progesterone receptor. *Conclusion*: PTEN protein expression was decreased in well-differentiated and less growth-aggressive endometrial carcinoma with wild-type p53 gene and high levels of ER and PR. This suggests that disturbed PTEN expression occurs in an early phase of the tumorigenesis of well-differentiated endometrial carcinoma.

Keywords PTEN · p53 · Estrogen receptor · Progesterone receptor · Endometrioid adenocarcinoma

F. Kimura · T. Fujisawa · Y. Kamata · Y. Nishimura
H. Kuramoto
Department of Clinical Cytology,
Graduate School of Medical Sciences,
Kitasato University, Kitasato, Kanagawa, Japan

J. Watanabe (✉)
Department of Pathology,
Kitasato University School of Medicine,
1-15-1, 228-8555 Kitasato, Sagamihara, Kanagawa, Japan
E-mail: watajun@med.kitasato-u.ac.jp
Tel.: +81-42-7789276
Fax: +81-42-7789276

H. Hata · T. Jobo
Department of Obstetrics and Gynecology,
Kitasato University School of Medicine,
Kitasato, Kanagawa, Japan

Introduction

The tumor suppressor gene PTEN (phosphatase and tensin homologue deleted on chromosome 10) is localized on chromosome 10q23. The gene product is a 55-kD protein composed of 403 amino acids. PTEN is a dual-specificity phosphatase with a sequence similar to that of the cytoskeletal protein tensin (Hinoda et al. 1998; Maehama and Dixon 2000; Parsons 1998; Tamura et al. 1999). PTEN is also frequently mutated in a wide range of human tumors such as glioblastoma (Sano et al. 1999; Steck et al. 1997) and cancers of the prostate (Girl and Ittamann 1999), breast (Perren et al. 1999), thyroid (Gimm et al. 2000), ovary (de la Cuesta et al. 1996; Obata et al. 1998) and endometrium (Ellenson 2000; Mutter et al. 2000b). Most of the mutations of the PTEN gene in tumors are localized in the phosphatase