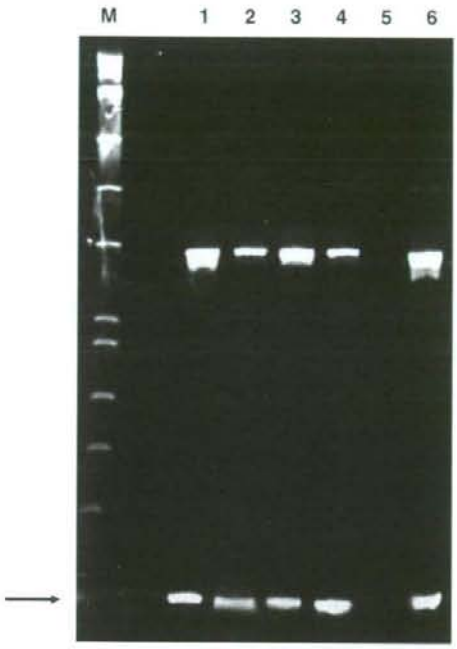

Fig. 4. Effect of epigallocatechin gallate (EGCG) and/or retinoic acid (RA) on the expression of hTERT mRNA in HeLa (lanes 1 and 2) and TMCC-1 (lanes 3 and 4). hTERT expression levels were quantified by a real-time PCR system. Lanes 1 and 3: no treatment; lanes 2 and 4: 1 μM of RA and 100 μM of EGCG; lane 5: negative control; lane 6: positive control (HeLa). The arrow indicates the PBDG.

results are consistent with those of telomerase activity by stretch PCR assay.

Discussion

It has been suggested that cervical adenocarcinoma has relatively more aggressive biological activity, poor prognosis, earlier metastasis and less sensitivity to radiation and chemotherapy, compared to squamous cell carcinoma [3,4]. It is necessary to develop a new anticancer agent for treatment of cervical adenocarcinoma. Polyphenols derived from green tea, particularly EGCG, have been reported to control the proliferation of various cancers and possess the anticarcinogenic and chemopreventive effects in various cancers both in vitro and in vivo [5–7,9–11,17–19]. Previously, we reported that EGCG prevented the carcinogenesis of cervical cancer [12]. It is also reported that the inhibitory effect was less in cervical adenocarcinoma cell lines than squamous cell carcinoma cell lines [12]. EGCG may be effective for treatment of cervical cancer, but, alone, it is inadequate for cervical adenocarcinoma.

Recently, Tachibana et al. [14] reported that all-trans-retinoic acid (RA) enhanced the binding of EGCG to the cell surface of cancer cells. It is also revealed that the cell surface 67-kDa laminin receptor (67 LR) is the target for EGCG and acts as the receptor for antitumor action of EGCG. The growth inhibitory activity of EGCG correlated with the binding strength of EGCG to the cell surface. Our previous study showed that RA EGCG inhibited cell growth for cell lines derived from the endocervix and cervical adenocarcinoma generally less effectively than those derived from their squamous cell counterparts [12]. In the present study, we investigated the efficacy of EGCG and/or RA in cervical adenocarcinoma cells. Our study showed that the combination treatment of EGCG and RA increased the antiproliferative effect in cervical adenocarcinoma cell lines. It is reported that the expression of 67 LR was enhanced by RA treatment [14]. The enhancement of 67 LR by RA treatment may result in the combination effect of EGCG and RA.

The mechanism of cancer inhibition of EGCG is not clear, but several hypotheses have been proposed [20]. It has been reported that EGCG induced apoptosis, and G1 or G2-M arrest of the cell cycle [13,17,21–25]. We previously reported that EGCG treatment resulted in DNA ladder formation in the cell lines from squamous cell, but not the adenocarcinoma counterparts [12]. EGCG treatment induced DNA ladder formation in HPV-18 immortalized endocervical cells, but not in serum adapted HPV-18 immortalized endocervical cells, which confers greater cervical cell growth potential and higher grade cervical lesion [12]. These results supported the hypothesis that the sensitivity to EGCG and induction of apoptosis by EGCG in the carcinogenesis of cervical adenocarcinoma decreases according to the progression to the carcinogenic process. The present study demonstrated that the combination treatment of EGCG and RA induced apoptosis in adenocarcinoma cells. EGCG or RA treatment alone, which was less effective in growth inhibition, did not induce apoptosis. We previously found that RA did not induce apoptosis [26]. These results suggest that the induction of apoptosis by the combination treatment of EGCG and RA is one of the important mechanisms for the EGCG-mediated anticancer effects in cervical adenocarcinoma. The induction of 67 LR by RA may be associated with the induction of apoptosis thorough the enhancement of the binding strength of EGCG to the cell surface. Although the mechanism of EGCG-induced apoptosis is not clear, the activation of caspases has been shown in cancer cells treated with EGCG [27–29]. In the present study, caspase-3 activity was induced by combination treatment of EGCG and RA. These results were consistent with those of an antiproliferative effect of EGCG and/or RA in adenocarcinoma cells.

The activation of telomerase has been proposed to be a critical event in the immortalization of human cells and is characteristic of most human cancer cell lines and tumors, including cervical cancer carcinogenesis [30–32]. It is reported that telomerase inhibition could be one of the major mechanisms in the anticancer effects of EGCG [20,33–36]. Previously, we reported that EGCG treatment inhibited telomerase activity in immortalized cervical cell lines, as well as non-transformed, serum-adapted HPV-18 immortalized endocervical cell lines and transformed HPV-18 immortalized ectocervical cell lines [12]. In this study, we demonstrated that EGCG treatment did not inhibit telomerase activity in cervical adenocarcinoma cells, but the combination treatment of EGCG and RA did. The effect was associated with a decrease of the hTERT expression.

With regard to in vivo studies, EGCG has been reported to prevent the formation of various solid tumors. The effective EGCG

levels were lower than those of in vitro models. The concentration of EGCG shown to have an effect in these previous in vitro studies (10–200 μM) is much higher than those observed in the blood or tissue after drinking tea [37], although the concentration of RA, 1 μM, was a peak plasma level of oral retinoid therapy. There is the disparity between the concentrations needed to achieve the various effects observed in vitro and the plasma levels at which significant anticancer and chemopreventive effects were observed in animal and epidemiological studies. Several mechanisms of cancer inhibition by EGCG in vivo have been proposed [38–40]. It is reported that EGCG inhibited angiogenesis and matrix metalloproteinase in vivo [38,40]. Recently, Tachibana et al. [14] reported that the 67-kDa laminin receptor (67 LR) was associated with EGCG responsiveness to cancer cells at physiologically relevant concentrations. It is also reported that the combination of EGCG and vitamin A increased the expression of the 67 LR that mediates the anticancer activity of EGCG and enhances the anticancer activity of EGCG. Vitamin A in plasma may result in lower levels of effective EGCG in animal and epidemiological studies. Considering our results and these reports, the combination treatment of EGCG and RA could therefore be a promising strategy in treatment for cancer, including cervical adenocarcinoma.

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Uterine Artery Embolization Followed by Dilation and Curettage for Cervical Pregnancy

Yoshifumi Nakao, MD, Masatoshi Yokoyama, MD, and Tsuyoshi Iwasaka, MD

BACKGROUND: Cervical pregnancy can be a life-threatening condition due to the risk of severe hemorrhage. Progression of ultrasonographic diagnostic technology has allowed the early detection of cervical pregnancy. However, a standard treatment protocol for fertility preservation has not yet been established.

CASE: Two women with cervical pregnancy presented with cardiac activity at 6 and 7 weeks of gestation. They were treated with transfemoral uterine artery embolization followed by dilation and curettage with minimal bleeding. One patient gave birth to a healthy neonate 20 months after the procedure.

CONCLUSION: Early cervical pregnancies were treated with dilation and curettage after uterine artery embolization. This treatment can be considered as conservative management for patients who desire to preserve their fertility.

(Obstet Gynecol 2008;111:505–7)

Cervical pregnancy can be a life-threatening condition due to the risk of severe hemorrhage. Cervical pregnancy is the implantation of a developing conceptus in the endocervical mucosa. Diagnosis and treatment of cervical pregnancy has changed dramatically in recent decades. Before 1980, the diagnosis was commonly made when dilation and curettage (D&C) for presumed incomplete abortion resulted in unexpected catastrophic hemorrhage. Emergency hysterectomy usually ensued.[1] Cervical pregnancy is now commonly diagnosed on a routine transvaginal ultrasound tomography examination in first-trimester pregnant patients without bleeding. However, a protocol for conservative management in early cervical pregnancy patients has not been established, as yet.

Below, we present two cases of cervical pregnancy in which uterine artery embolization was performed as a prophylactic procedure before D&C. According to our experience and review of published reports written about the management of cervical pregnancy, new suggestions are made regarding the potential role of uterine artery embolization before D&C in the treatment of cervical pregnancy.

CASE 1

A 27-year-old, gravida 2, para 0, with an ultrasonographic diagnosis of cervical pregnancy at the sixth week of gestation was referred to our hospital in 2005. She presented with painless fresh vaginal bleeding. Transvaginal ultrasonography showed a yolk sac with a positive fetal heart beat in a 13.2-mm-sized gestational sac located within the cervical canal (Fig. 1.). The urine human chorionic gonadotropin (hCG) was 3,951 milli-International Units/mL. Her vital signs were stable, and findings from a general physical examination were unremarkable. In the uterine cavity, there were no endometrial signs of an intrauterine pregnancy. As the patient expressed a strong desire to preserve her fertility and presented a moderate amount of flesh uterine bleeding, conservative management with uterine artery embolization followed by D&C was planned with informed consent. Under fluoroscopic control, each uterine artery was embolized with minced gel foam. After the procedure, uterine bleeding was immediately decreased. To avoid severe postembolization ischemic pain, continuous epidural analgesic reagent injection was started before uterine artery embolization. Twenty-four hours after the uterine artery embolization, D&C of the cervical pregnancy was performed under general anesthesia. The estimated blood loss was 20 mL. After the evacuation, no uterine bleeding occurred. Three days after D&C, a ultrasound examination showed a normal structure of the uterine cervix and body. Pathological examination of the intracervical curettage specimen confirmed the products of conception. The urinary hCG titer was decreased to normal range within 4 weeks. Seventy-six days after the procedure, the patient resumed regular menstrual cycles.

A natural intrauterine pregnancy was confirmed 12 months after the procedure. The course of pregnancy was unremarkable except the patient received prophylactic cervical cerclage for suspected cervical incompetency with a wedge-shaped cervix detected by transvaginal ultrasound tomography at 19 weeks of gestation on routine screening. At 36 weeks of gestation, her membranes ruptured spontaneously, the cerclage was removed, and she gave birth to a healthy neonate after a spontaneous labor. The newborn (male, weight 3,016 g, Apgar scores 8 and 9) and the mother's course were uneventful after delivery.

From the Department of Obstetrics and Gynecology Faculty of Medicine, Saga University, Saga City, Saga, Japan.

Corresponding author: Yoshifumi Nakao, MD, Department Obstetrics and Gynecology, Faculty of Medicine, Saga University, Saga City, Saga, 849-8501, Japan; e-mail: nakaoy@cc.saga-u.ac.jp.

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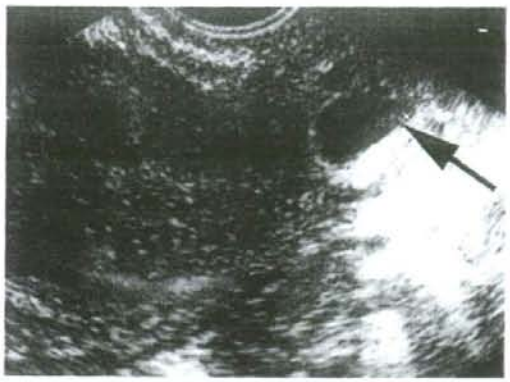

Fig. 1. Sagittal transvaginal ultrasonographic view of the uterus in case 1 showed gestational sac in the cervical canal. York sac was also noted *(arrow).*

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CASE 2

A 41-year-old, gravida 2, para 1 with a sonographic diagnosis of cervical pregnancy at the seventh week of gestation was referred to our hospital in 2006. The vaginal examination revealed no uterine bleeding. The transvaginal ultrasonography demonstrated a gestational sac and a fetus with a positive fetal heartbeat located in the mid portion of the endocervical canal. The crown–rump length was 12 mm, corresponding to 7 weeks of gestation. The patient expressed a strong desire to maintain her fertility. After providing informed consent, she agreed to undergo conservative treatment with uterine artery embolization followed by D&C. At day 1, angiographic embolization of the bilateral uterine arteries was successfully performed with gel foams under continuous epidural anesthesia using fentanyl and ropivacaine hydrochloride hydrate. On the following day, D&C was carried out without unexpected bleeding. The urinary hCG titer was 25,700 milli-International Units/mL on the day of the operation. Within 48 hours, the urinary hCG decreased to 3,340 milli-International Units /mL, and the patient was discharged. Histological examination of the curettaged specimen confirmed the products of conception. She resumed a normal menstruation cycle 4 weeks later.

COMMENT

Various conservative methods for cervical pregnancy termination have been suggested in an attempt to avoid hemorrhage, preserve the uterus and maintain fertility, such as D&C followed by intracervical tamponade, cervical cerclage, or operative ligation of uterine arteries, and antitrophoblastic chemotherapy.[1]

Dilation and curettage with subsequent tamponade of the defect in the cervical canal used to be the orthodox method for management of cervical pregnancy. Various procedures have been tried to avoid uncontrollable massive postevacuation bleeding. For many years, cervical or vaginal packing with gauze has been the primary method used to control such bleeding. As the packing device, a Foley catheter was also used. Other methods for local control are transvaginal ligation of the descending branches of the uterine arteries, cervical cerclage, and intracervical injection of vasoconstrictors like vasopressin or prostaglandins.[2] However, these procedures showed uncertainty.

Systemic and/or local instillation of methotrexate (MTX) has become one of the main choices among fertility-preserving therapies for cervical pregnancy because it is convenient to perform and has a high success rate in selected cases. Since Oyer et al[3] reported the first successful case of cervical pregnancy treated with MTX, many patients have been treated by various systemic or local chemotherapeutic agents or both. Most cases were treated with an MTX protocol similar to those used in gestational trophoblastic disease.[1,4] During the treatment, unexpected massive bleeding was sometimes observed, and additional procedures were necessary for hemostasis. The effectiveness of MTX for the treatment of cervical pregnancy is reduced with β-hCG levels of greater than 10,000 milli-International Units/mL, gestational age greater than 9 weeks, and positive fetal heart activity.[5] Kim et al[6] reported that concomitant procedures with the MTX treatment were required in 54% of cases with positive fetal heart beat and in 50% of cases of greater than 6 weeks of gestation in their series. These results suggest that MTX treatment cannot be considered a criterion standard for the treatment of cervical pregnancy.

Bilateral internal iliac artery ligation is performed in patients refractory to local treatment after evacuation of cervical pregnancy. However, the high complication rate of general anesthesia and emergency surgery in patients who are already hemodynamically unstable should be taken into consideration. Recently, uterine artery embolization has been shown to be effective for the treatment of acute pelvic hemorrhage and obstetric emergencies including postpartum hemorrhage, postabortion hemorrhages with placenta accrete, and cervical pregnancy. Uterine artery embolization is now widely accepted as an alternative treatment for uterine fibroids. Although rare serious complications related to uterine artery embolization for fibroids are presented in recent case reports, including endometrial atrophy with permanent amenorrhea, uterine necrosis, and fatal sepsis, cases of

pregnancy after uterine artery embolization even for uterine fibroids have recently been reported. As to the treatment tool for cervical pregnancy, uterine artery embolization was initially used after surgical evacuation to reduce postoperative blood loss and preserve fertility, instead of surgical internal iliac artery ligation. Uterine artery embolization was secondarily used when uncontrollable bleeding occurred after chemotherapy. Recently, prophylactic use of uterine artery embolization before D&C was also applied to avoid massive intraoperative bleeding.[7,8] We experienced two successful cases of patients treated by uterine artery embolization before D&C, one of which was followed by a successful pregnancy without adverse events. Furthermore, for both cases, no additional chemotherapy was needed. This conservative method should be applied at least during the first trimester because the blood flow of the ovarian arteries will increase in the second trimester. Therefore, early and correct diagnosis of cervical pregnancy is necessary.

In conclusion, D&C after uterine artery embolization was shown to be effective for patients with cervical pregnancy during the first trimester, especially for those with massive vaginal bleeding. This therapy has potential to minimize the patient's discomfort and recovery time to and preserve fertility.

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Clusterin expression predicts survival of invasive cervical cancer patients treated with radical hysterectomy and systematic lymphadenectomy

Hidemichi Watari [a,*], Yoko Ohta [a], Mohamed Kamel Hassan [a], Ying Xiong [a], Shinya Tanaka [b], Noriaki Sakuragi [a]

[a] Department of Obstetrics and Gynecology, Hokkaido University Graduate School of Medicine, Sapporo, 060-8638, Japan
[b] Department of Molecular Cytopathology, Hokkaido University Graduate School of Medicine, Sapporo, 060-8638, Japan

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Abstract

Objectives. The aim of this study was to evaluate the prognostic significance of clusterin expression in invasive cervical cancer patients treated with radical hysterectomy and systematic lymphadenectomy.

Methods. Invasive cervical cancer specimens were obtained from 52 patients who underwent radical hysterectomy and systematic lymphadenectomy at Hokkaido University Hospital from 1997 to 2004. The expression of clusterin protein was analyzed by immunohistochemical staining. Findings were evaluated in relation to several clinicopathological factors. Survival analyses were performed by the Kaplan–Meier curves and the log-rank test. Independent prognostic factors were determined by multivariate Cox regression analysis.

Results. Clusterin protein was present in the cytoplasm of cervical cancer cells. The expression of clusterin protein in invasive cervical cancer tissues was not related to any clinicopathologic factors analyzed. Patients with positive clusterin expression showed significantly worse prognosis than those with negative clusterin expression ($p=0.017$). Multivariate analysis including clusterin expression revealed that clusterin expression ($p=0.006$) and the number of positive node groups ($p=0.002$) were independent prognostic factors for survival. Survival of patients with invasive cervical cancer could be stratified into three groups by combination of clusterin expression and number of positive node groups with an estimated 5-year survival rate of 100.0% for no or one positive node group irrespective of clusterin expression (group A), 78.7% for multiple node groups with negative clusterin expression (group B), and 14.3% for multiple node groups with positive clusterin expression (group C) ($p=0.03$ for group A vs. group B, $p=0.004$ for group B vs. group C, and $p<0.0001$ for group A vs. group C).

Conclusions. Clusterin expression and the number of positive node groups were independent prognostic factors for invasive cervical cancer patients treated with radical hysterectomy and systematic lymphadenectomy. Clusterin might be a new molecular marker to predict the survival of cervical cancer patients with multiple positive node groups.

Keywords: Clusterin; Cervical cancer; Prognostic factor; Lymphnode metastasis; Immunohistochemistry

Introduction

Cervical carcinoma is generally treated by surgery, radiotherapy or both. At many institutions in Japan, cervical carcinoma ((FIGO stage Ib through IIb) is treated with radical surgery followed by adjuvant radiotherapy when risk factors for recurrence, including lymph vascular space invasion (LVSI), deep stromal invasion (DSI), parametrial invasion (PI) and lymph node metastasis (LNM) are found by postoperative pathological examinations. We have performed type IV radical hysterectomy with systematic lymphadenectomy [1,2], and previously reported that patients with multiple positive node groups showed significantly worse prognosis than those with no or one positive node group in cervical cancer patients treated with radical hysterectomy and systematic lymphadenectomy [3]. After the extensive surgery, the efficacy of adjuvant therapy is

* Corresponding author. Department of Obstetrics and Gynecology, Hokkaido University Graduate School of Medicine, North 15, West 7, Kita-Ku, Sapporo, 060-8638, Japan. Fax: +33 81 11 706 7711.
E-mail address: watarih@med.hokudai.ac.jp (H. Watari).

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one of the most important factors to improve the survival. The molecules, which are implicated in the mechanism of chemosensitivity or radiosensitivity, could be good candidates for new molecular markers to predict survival of cancer patients who receive adjuvant therapy.

Clusterin, also known as testosterone-repressed prostate message-2, sulphated glycoprotein-2 or apolipoprotein J, is a heterodimeric highly conserved disulfide-linked glycoprotein that is expressed in a wide variety of tissues and secreted in all human fluids and is implicated in various pathophysiological processes, including lipid transport, sperm maturation, complement inhibition, tissue remodeling, cell differentiation and transformation, and apoptotic cell death [4]. It has been found to be overexpressed in several types of malignant tumors, and in these cancers, clusterin overexpression has been reported to be closely associated with cancer progression [5–8]. Furthermore, the antiapoptotic action of clusterin has been reported to increase drug resistance [10–14] and radioresistance [15–17]. Clusterin expression has been associated with clinicopathologic parameters of aggressiveness and unfavorable prognosis [7,18–21]. Taken together, these reports indicate that clusterin may play an important role in chemoresistance, radioresistance and may be a potent prognostic biomarker for different tumor types.

The aim of this study was to investigate the expression of clusterin by immunohistochemistry in invasive cervical cancer and the potential correlation between clusterin expression and clinicopathological parameters, and the prognostic significance of clusterin expression in cervical cancer.

Materials and methods

Tissue specimens

Tumor samples were obtained from 52 invasive cervical cancer patients treated with radical hysterectomy and lymphadenectomy at Hokkaido University Hospital from 1997 to 2004. All tissue samples were examined pathologically by two pathologists. The median follow-up of the patients was 48 months (range; 6 to 104 months). The median age of the patients was 46 years (range; 28 to 71).

Immunohistochemistry

Immunohistochemical analysis was performed on 4 μm paraffin sections, which were mounted on SuperFrost Plus slides, deparaffinized and rehydrated through xylene and alcohol. Endogeneous peroxidase was blocked in 3% H_2O_2, for 30 min and heat-induced epitope retrieval was performed using citrate buffer, pH 6.0. Detection of clusterin was performed using a commercial polyclonal antibody (clusterin alpha/beta rabbit polyclonal antibody H330: Santa Cruz Biotechnology, Santa Cruz, CA, USA). The clusterin antibody was used at 1:200 dilution for overnight at 4 C. After washing in Tris-buffered saline, the sections were incubated with the Envision detection system for 30 min. Staining was completed after 15 min of incubation with a freshly prepared substrate–chromogen solution. Sections were washed in distilled water and lightly counterstained with haematoxilin, followed by rehydration and coverslip mounting. Negative control was obtained by omitting the primary antibody. All slides were blindly evaluated for clusterin immunoreactivity and protein localization, without knowledge of clinicopathological data.

Staining intensity was scored from 1+ (negative), 2+ (weak), 3+ (moderate), to 4+ (strong), and staining extent was also scored from 1+ (<10%), 2+ (10–25%), 3+ (26–50%) to 4+ (>50%) as previously described with some modification [22]. The sum of staining intensity and staining extent was defined as the staining score.

Statistics

Correlation between the variables and staining score of clusterin immunoreactivity was analyzed using chi square test. Patients survival was calculated using Kaplan–Meier method. The significance of the survival difference was examined by the log-rank test. Univariate and multivariate survival analyses were performed using the Cox regression model with disease-specific overall survival as the outcome measure. Forward stepwise procedure was used to select the independent variable in multivariate analysis. $p<0.05$ was considered statistically significant. Statistical analyses were performed with the Statview software package version 5.0 (SAS Institute, Inc, Cary, NC).

Results

Immunohistochemical expression of clusterin and its association with clinocopathological factors in patients with cervical cancer

The clinical characteristics of the patients are listed in Table 1. 20 patients had stage Ib disease, 5 stage IIa and 27 stage IIb. The following histological tumor types were found; 35 squamous cell carcinomas, 3 adenosquamous carcinomas, 14 pure adenocarcinomas (including 8 endocervical type, 4 endometrioid, 2 clear cell). 20 patients had negative node group, 14 one positive node group, and 18 multiple positive node groups. 44 patients (84.6%) received adjuvant therapy (radiotherapy or chemotherapy or both).

Immunolocalization with anti-clusterin antibody largely showed positive staining in the cytoplasm of cancer cells and occasionally positive in the nucleus. Representative results of staining intensity were shown in Fig. 1. Normal squamous epithelium revealed negative staining, but occasionally positive in normal epithelium of endocervical glands (data not shown).

The association between the staining of clusterin protein in invasive cervical cancer tissues and several clinicopathological factors revealed that the expression of clusterin protein was not significantly related to any variables, including age ($p=0.62$), FIGO stage $p=0.26$), histologic subtype ($p=0.054$), blood

Table 1
Clinicopathological characteristics of invasive cervical cancer

Variables	*n*	%
FIGO stage		
Ib	20	38.5
IIa/IIb	32	61.5
Histologic sub type		
squamous/adenosquamous	38	73.1
pure adeno	14	26.9
Vascular invasion		
negative	35	67.3
positive	17	32.7
Ovarian metastasis		
negative	50	96.2
positive	2	3.8
Number of positive node group		
none or one node group	34	65.4
multiple node groups	18	34.6
Adjuvant therapy		
(−)	8	15.4
(+)	44	84.6

vessel invasion ($p=0.49$), ovarian metastasis ($p=0.16$), and the number of positive node groups ($p=0.87$) (Table 2).

Prognostic impact of clusterin expression

Fig. 1 shows the survival of patients with invasive cervical cancer according to the staining score of clusterin expression.

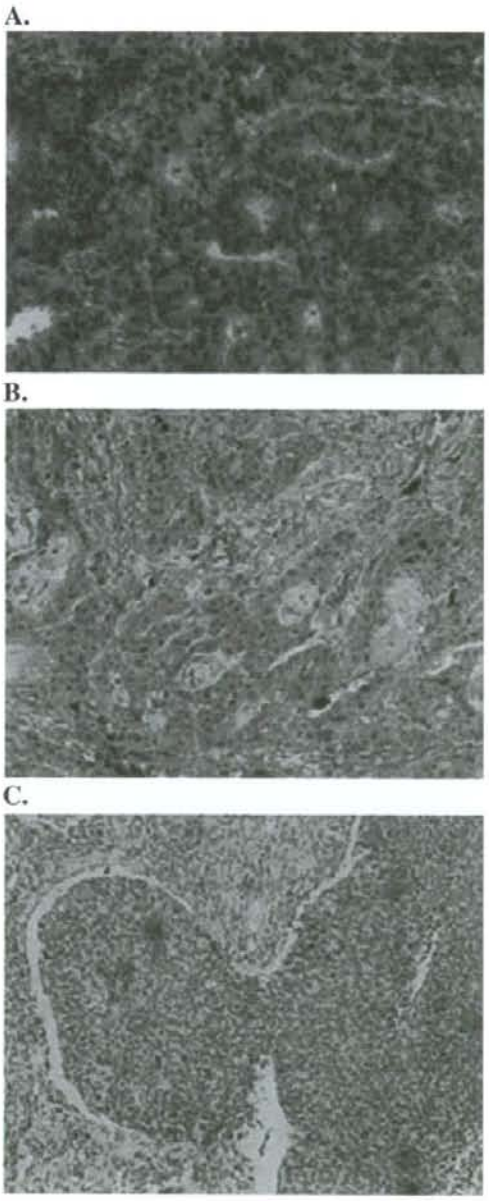

Fig. 1. Typical outcomes of immunohistochemical staining of clusterin expression in cervical cancer tissues. (A) Cervical cancer with strong clusterin staining (×100), (B) cervical cancer with weak clusterin staining (×100), (C) cervical cancer with negative clusterin staining (×50).

Table 2
Correlation between clusterin expression and clinicopathological factors in invasive cervical cancer

Variables	Clusterin immunoreactivity		*p* value
	Score (≤3)	Score (≥4)	
Age			0.62
<40	7	6	
>=40	24	15	
FIGO stage			0.26
Ib	10	10	
IIa/IIb	21	11	
Histologic subtype			0.05
squamous/adenosquamous	26	12	
pure adeno	5	9	
Vascular invasion			0.49
negative	22	13	
positive	0	2	
Ovarian metastasis			0.16
negative	31	19	
positive	0	2	
Number of positive node group			0.87
none or one node group	20	14	
multiple node groups	11	7	

The estimated 5-year survival rate was 93.2% for patients with the staining score of <=3 ($n=31$), 71.4% for those with the staining score of >=4 ($n=21$). There was statistically significant difference between the group with the staining score of <=3 and that with the score of >=4 ($p=0.017$), indicating that the clusterin expression could be a new prognostic marker for invasive cervical cancer (Fig. 2).

Univariate and multivariate survival analysis of the patients with invasive cervical cancer

Since the staining score of clusterin expression was shown to have significant impact on the survival of patients with invasive cervical cancer who underwent radical hysterectomy and systematic lymphadenectomy, we included the staining score of clusterin expression in the multivariate analysis. The univariate

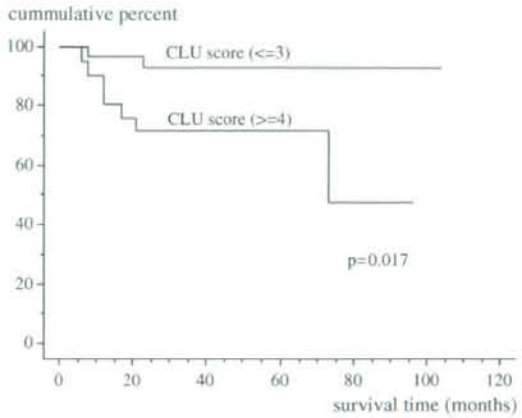

Fig. 2. Overall survival of patients with cervical cancer who underwent radical hysterectomy and systematic lymphadenectomy according to clusterin expression.

Table 3
Univariate and multivariate Cox regression analysis of prognostic factors of cervical cancer

Univariate		Multivariate		
Clinicopathologic factor	p value	Risk ratio	95% CI	p value
age	0.67	–	–	NS
FIGO stage	0.29	–	–	NS
histologic subtype	0.002	–	–	NS
blood vessel invasion	0.002	–	–	NS
ovarian metastasis	<0.0001	–	–	NS
lymphnode metastasis	<0.0001	50.0	4.8–500	0.001
clusterin immunoreactivity	0.02	11.8	2.0–66.7	0.006

NS: not significant.
FIGO stage: stage Ib vs. II.
Histologic subtype: squamous/adenosquamous vs. pure adeno.
Blood vessel invasion: negative vs. positive.
Ovarian metastasis: negative vs. positive.
Lymph node metastasis: negative/ one positive node group vs. multiple positive node groups.
Clusterin immunoreactivity: staining score (≤ 3) vs. (≥ 4).

analysis revealed that the histologic subtype (endocervical adenocarcinoma) ($p=0.002$), blood vessel invasion ($p=0.002$), ovarian metastasis ($p<0.0001$), number of positive node group ($p<0.0001$), and clusterin expression ($p=0.02$) were shown to be related to poor survival. Age ($p=0.67$), and FIGO stage ($p=0.29$) were not related to poor survival in this cohort (Table 3, Fig. 3).

Multivariate analysis, including the prognostic factors determined by univariate analysis to have statistical significance, was performed using a forward stepwise procedure. It revealed that number of positive node group ($p=0.001$) and clusterin expression ($p=0.006$) were independent prognostic factors (Table 3). Survival of patients with invasive cervical cancer could be stratified into three groups by combination of number of positive node group and clusterin expression with an estimated 5-year survival rate of 100.0% for none or one positive lymph node group irrespective of clusterin expression (group A), 78.7% for >=2 positive lymph node groups with lower clusterin expression (group B), and 14.3% for >=2 positive lymph node groups with higher clusterin expression (group C), whose estimated survival did not reach 5 years yet (43 months). The difference of survival rate between each group was statistically significant ($p=0.03$ for group A vs. group B, $p=0.004$ for group B vs. group C , $p<0.0001$ for group A vs. group C) (Fig. 3).

Discussion

In the present study, we found that clusterin might be a new molecular marker to predict survival of invasive cervical cancer patients treated with radical hysterectomy and systematic lymphadenectomy. Although Park et al. firstly reported overexpression of clusterin in cervical cancer tissues compared with normal squamous epithelium of the cervix by immunohistochemistry [23], they failed to show prognostic significance of clusterin expression in their cohort, probably because of small number of cases. Thus, this is the first report on the prognostic significance of clusterin in cervical cancer as far as we know. We found more cases with negative staining of clusterin than the first report [23] in our cohort. This is at least, in part, due to difference of the antibody used, difference of specimens (frozen vs. formalin-fixed). The antibody we used in this study, however, has been widely used for immunostaining on the formalin-fixed tissue sections of other cancer types [9,12,14,22]. We, therefore, might get more cases with stronger staining using frozen tissue sections of cervical cancer. We also need to further investigate the clusterin expression in cervical cancer by quantitative method and correlate with pathologic factors because we used immunohistochemistry, a non-quantitative method, to evaluate the expression of clusterin in this study.

Prognostic significance of clusterin expression have been reported in other cancer types. The level of expression of clusterin in renal cell cancer was found to be closely associated with pathological stage and grade of the tumor, and the overall and recurrence-free survival rate of patients with strong clusterin expression was significantly lower than that of patients with weak expression [19]. Clusterin expression levels correlated with tumor size, estrogen and progesterone receptor expression levels, and lymph node metastasis in breast carcinoma [7]. Clusterin has been proposed to be a potentially new prognostic and predictive marker for colon carcinoma aggressiveness, since overexpression of clusterin is observed in highly aggressive tumors as well as metastatic nodules [8].

The exact reason why clusterin expression showed prognostic significance for cervical cancer remains to be elucidated. Clusterin has been shown to have antiapoptotic function in other cancer types. In those tumor types, knockdown of clusterin expression by antisense oligonucleotide or Si-RNA sensitized cancer cells to chemotherapeutic agents such as paclitaxel, cisplatin [10–14]. Clusterin also has been implicated in resistance to radiotherapy in lung, prostate, and bladder cancer [15–17]. Park et al. showed a significant correlation between clusterin expression and paclitaxel IC50 in the cervical cancer cell lines and cervical cancer cells expressing high levels of

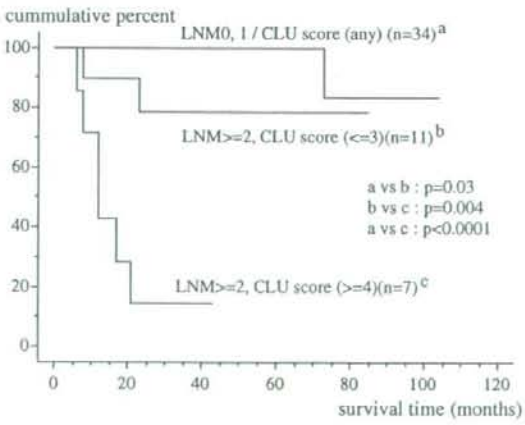

Fig. 3. Overall survival of patients with cervical cancer who underwent radical hysterectomy and systematic lymphadenectomy by the combination of clusterin expression and number of positive node groups.

clusterin became more sensitive to paclitaxel after silencing clusterin with Si-RNA, suggesting that clusterin may play a role in antagonizing the antitumor activity of paclitaxel in cervical cancer cells [23]. We have also found that clusterin expression was upregulated in paclitaxel- resistant ovarian cancer cells compared with parental sensitive cells and knockdown of clusterin expression with Si-RNA sensitized them to paclitaxel in vitro [24]. Taken together, these findings indicate that clusterin might be involved in the general mechanism of resistance to paclitaxel in cancer cells. We also speculate that clusterin is one of the key molecules to determine the sensitivity to adjuvant therapy (radiotherapy, chemotherapy) in cervical cancer cells. We, therefore, need to further examine the role of clusterin in resistance to radiotherapy, which is one of the primary therapy for cervical cancer, and in resistance to other chemotherapeutic agents such as platinum using cervical cancer cell lines.

We have also shown that clusterin expression and the number of positive node groups are independent prognostic factors and clusterin expression might predict survival of node-positive patients with invasive cervical cancer (Fig. 3). It has been suggested that survival of patients with lymphnode metastasis may be improved by systematic lymphadenectomy and postoperative radiotherapy if the affected node is limited to one node group [25,26]. We have previously reported that patients with multiple positive node groups showed significantly worse prognosis than those with no or one positive node group in cervical cancer patients treated with radical hysterectomy and systematic lymphadenectomy [1,3]. To improve the outcome of node-positive patients, especially patients with multiple positive node groups, clinicians have searched for alternative adjuvant treatments such as chemotherapy [27–29]. In a randomized study, however, it was shown that selecting patients for adjuvant chemotherapy only on the basis of positive pelvic nodes did not result in an improvement of survival [30]. The reason for this might be that among the node-positive group, some patients have a good prognosis whereas others have a poor outcome. To enhance the efficacy of adjuvant radiotherapy or chemotherapy in node-positive patients, it is necessary to identify the molecules which induce resistance to chemotherapy or radiotherapy. Resistance to adjuvant therapies and disease recurrences could reliably be predicted by assessing biochemical factors strictly related to tumor cell biology and tumor aggressiveness, such as oncogenes and tumor suppressor genes. The result obtained in this study suggests that clusterin is one of the key molecules to induce resistance to adjuvant therapy in cervical cancer cells, which might result in poor survival of patients with positive clusterin expression.

Recent preclinical studies provide proof-of-principle evidence that targeting cell survival genes, such as clusterin or Bcl-2, with antisense oligonucleotides enhances apoptosis induced by conventional chemotherapy [31,32], and has led to clinical testing of antisense oligonucleotide therapy in combination with chemotherapy [33,34]. We, therefore, might combine the drug to knockdown clusterin expression such as OGX-011, the second generation of the clusterin antisense oligonucleotide, or Si-RNA, with cytotoxic agents or irradiation in the adjuvant setting for cervical cancer patients to enhance the efficacy of adjuvant therapy and to improve patients' survival in the near future.

In summary, we conclude that clusterin might be a new molecular marker to predict survival of cervical cancer patients, and that combination of adjuvant therapy with drugs targeting clusterin expression might be a new treatment strategy to improve poor survival of patients with multiple positive node groups in cervical cancer.

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Treatment of cervical cancer with adjuvant chemotherapy versus adjuvant radiotherapy after radical hysterectomy and systematic lymphadenectomy

Masayoshi Hosaka, Hidemichi Watari, Mahito Takeda, Masashi Moriwaki, Yoko Hara, Yukiharu Todo, Yasuhiko Ebina and Noriaki Sakuragi

Department of Obstetrics and Gynecology, Hokkaido University Graduate School of Medicine, Sapporo, Japan

Abstract

Aim: To compare the clinical efficacy focused on post-treatment morbidity between adjuvant chemotherapy (CT) and pelvic radiotherapy (RT) after radical hysterectomy for patients with cervical cancer.

Methods: A total of 125 patients with cervical squamous cell carcinoma who underwent radical hysterectomy and pelvic lymphadenectomy at Hokkaido University Hospital between 1991 and 2002 were enrolled in the study for retrospective analysis. Seventy patients with recurrent risk factors, including deep stromal invasion, lymph vascular space invasion, parametrial invasion, lymph node metastasis (LNM), and bulky tumor (≥4 cm), received adjuvant therapy; 42 were treated with RT, and 28 were treated with CT. Almost all patients with multiple LNM received RT. Analyses were also performed on a subgroup of 50 patients without multiple LNM (23 RT, 27 CT). Clinical efficacy of post-treatment morbidity and survival was evaluated.

Results: Because there were more patients with multiple LNM in the RT group, we analyzed disease-free survival in 50 patients without multiple LNM. The 3-year disease-free survival rate was 82.6% with RT and 96.3% with CT ($P = 0.16$). Postoperative bowel obstruction was significantly more frequent in the RT group versus the CT ($P = 0.007$) and no-therapy ($P = 0.0026$) groups. Urinary disturbance was also more frequent in the RT group than in the CT ($P = 0.0016$) and no-therapy ($P = 0.089$) groups.

Conclusion: CT has the equivalent therapeutic effect as RT with fewer postoperative complications for patients with intermediate risks. A prospective randomized trial is needed to compare CT combined with radical hysterectomy and pelvic lymphadenectomy to RT or chemoradiotherapy.

Key words: adjuvant chemotherapy and radiotherapy, cervical cancer.

Introduction

Cervical cancer is generally treated by surgery, radiotherapy (RT), or a combination of the two. At many institutions in Japan, cervical cancer (International Federation of Gynecology and Obstetrics [FIGO] stage Ib1-IIb) is treated with radical surgery and adjuvant RT when postoperative pathological examinations reveal risk factors for recurrence, including deep stromal invasion (DSI), lymph vascular space involvement (LVSI), parametrial invasion (PI), lymph node metastasis (LNM), and bulky tumor (tumor diameter >4 cm [BT]). We traditionally treated radical surgery first, and used RT as an adjuvant therapy for patients with stage Ib1-IIb disease. However, there is no definitive evidence that RT is beneficial after radical surgery for cervical cancer.[1,2] Adjuvant chemotherapy (CT) combined with radical hysterectomy and systematic lymphadenectomy may also provide a survival benefit. However, there are no randomized

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Reprint request to: Dr Hidemichi Watari, Department of Obstetrics and Gynecology, Hokkaido University Graduate School of Medicine, North 15 West 7 Kita-ku, Sapporo, 060-8638, Japan. Email: watarih@med.hokudai.ac.jp

controlled studies comparing the clinical efficacy of CT and RT.

In our institution, adjuvant RT was used after radical hysterectomy. However, we observed severe postoperative complications (lymphedema, bowel obstruction and urinary disturbance) among patients receiving adjuvant RT that significantly reduced quality of life. Based on these observations and some data suggesting that the therapeutic effects of adjuvant CT and adjuvant RT were similar,[3] we began to use more frequent adjuvant CT after surgery after the year 2000. In the present retrospective study, we investigated the clinical efficacy of adjuvant RT and CT after radical and systematic lymphadenectomy in women with cervical cancer by comparing patient survival and postoperative complications.

Methods

One hundred twenty-five patients with FIGO stage Ib1-IIb cervical squamous cell carcinoma were treated at Hokkaido University Hospital between 1991 and 2002. All patients underwent radical hysterectomy with removal of a vaginal cuff of at least 2 cm, total resection of parametrial tissue and systematic retroperitoneal lymphadenectomy. This operation is a nerve-sparing modification of the Okabayashi operation.[4,5] The nerve-sparing procedure was further refined by introducing the preservation of vesical branches of pelvic plexus after 1997. Patients with risk factors for recurrence, including DSI (>2/3 thickness), LVSI, PI, LNM, and BT, received adjuvant RT or CT.

RT consisted of whole pelvic external irradiation by four-field technique with 50 Gy for 25 fractions beginning 4 weeks after surgery. Chemotherapy consisted of bleomycin (7 mg/body from days 1–5), vincristine (0.7 mg/m^2 on day 5), mitomycin C (7 mg/m^2 on day 5), and cisplatin (14 mg/m^2 from days 1–5). Patients received at least three courses at 4-week intervals beginning 2–3 weeks after surgery.

The patients' 3-year overall survival and disease-free survival were evaluated. We also assessed postoperative complications, including leg lymphedema, bowel obstruction and urinary disturbance. Patients with grade 1 leg edema were considered positive for lymphedema. Patients treated for bowel obstruction with intravenous infusion and/or surgery were considered positive for bowel obstruction. Patients with long-term self-catheterization or incontinence were considered positive for postoperative urinary disturbance.

Patient survival was calculated using the Kaplan–Meier method. The significance of the survival difference was evaluated using the log–rank test. The χ^2 test was used to analyze correlations between variables. Significance was set at $P < 0.05$. Statistical analyses were performed with the Statview software package (SAS Institute; Cary, NC, USA).

Results

Patient characteristics are listed in Table 1. Of the 125 patients who underwent radical hysterectomy, 83 patients had risk factors, 13 patients did not receive adjuvant therapy because four had post operative complications, and nine refused to receive adjuvant therapy. Forty-two of the patients with risk factors were treated with adjuvant RT between 1991 and 2000. In this group, the mean age was 50.3 years (range 28–74). Nine of the patients treated with adjuvant RT presented with clinical stage Ib1 cancer, 13 with stage Ib2 cancer, one with stage IIa cancer, and 19 with stage IIb cancer. Postoperative pathology examination confirmed that 24 patients had DSI, 35 had LVSI, 14 had PI, 16 had BT and 32 had LNM (19 cases had multiple LNM). Twenty-eight patients with risk factors for recurrence were treated with adjuvant CT between 2000 and 2002. Their mean age was 52.2 years (range 35–71). Eleven of these patients presented with clinical stage Ib1 cancer, nine with stage Ib2 cancer, two with stage IIa cancer, and six with stage IIb cancer. Postoperative pathology examination confirmed that 27 cases treated with adjuvant CT had some risk for recurrence; 11 had DSI, 27 had LVSI, five had PI, 10 had BT, and three had LNM. Only one case with multiple LNM received CT.

Treatment outcomes are shown in Table 2. In the RT group, 16 of 42 patients (38.1%) had recurrent disease and nine of 42 patients (21.4%) died due to disease of cancer. Seven patients had pelvic tumor recurrence affecting the vaginal stump, pelvic wall, or pelvic lymph node. Nine patients had extrapelvic recurrence affecting the lung, liver, brain, or supraclavicular lymph node. In the CT group, one of 28 patients (3.6%) experienced recurrence. The site of recurrence was the sacral surface, and the patient was alive with no evidence of disease after salvage radiation. The 3-year overall survival was 92.0% for the entire patient population, 98.2% for the patients not receiving adjuvant therapy, 100% for the CT group, and 78.5% for the RT group. The 3-year disease-free survival rate was 83.2% for the entire patient population, 92.7% for the patients

Table 1 Characteristics of 125 patients treated with radical hysterectomy and systematic retroperitoneal lymphadenectomy

	No treatment	Adjuvant therapy		*P*-value
		Radiotherapy	Chemotherapy	
No. patients	55	42	28	
Age	49.0 (28–76)	50.3 (28–74)	52.2 (35–71)	NS
Stage				NS
Ib1	26	9	11	
Ib2	5	13	9	
IIa	1	1	2	
IIb	23	19	6	
LVSI	13	35	27	NS
Depth of stromal invasion				NS
<2/3	49	18	17	
≥2/3	6	24	11	
Parametrial invasion	2	14	5	NS
Bulky tumor	0	16	10	NS
Pelvic lymph node metastasis				0.0001
0	54	10	25	
1	1	13	2	
≥2	0	19	1	

LVSI, lymph-vascular space invasion; NS, not significant.

Table 2 Outcome of 125 patients at 3 years after surgery, according to type of adjuvant therapy

Adjuvant therapy	No. patients	NED	Recurrence site (pelvic, distant)	DOD
No treatment	55	52	3, 0	1
Radiotherapy	42	26	7, 9	9
Chemotherapy	28	27	1, 0	0
Total	126	105	11, 9	10

DOD, dead of disease; NED, no evidence of disease.

not receiving adjuvant therapy, 96.4% for the CT group, and 61.9% for the RT group.

Because multiple LNM was more frequent in the RT group than in the CT group, we repeated the above analyses on 50 patients without multiple LNM. There was no statistically significant difference between the RT and CT patients with regard to clinical stage, DSI, LVSI, BT or PI. Single-node metastasis was more frequent in the RT group. The 3-year disease-free survival rate was 82.6% in the RT group and 96.3% in the CT group (Fig. 1); the difference was not statistically significant ($P = 0.16$).

The incidences of postoperative complications are shown in Table 3. Among the 55 patients who did not receive adjuvant treatment, eight (14.5%) had leg lymphedema, five (9.1%) had grade 1 bowel obstruction, and 12 (21.8%) had urinary disturbance. Among the 42 patients who received adjuvant RT, 12 (28.6%) had leg lymphedema, 16 (38.1%) had urinary disturbance, and 13 (31.0%) had bowel obstruction. Nine bowel obstructions were cured by conservative treatment, two required insertion of an ileus tube, and two necessitated intestinal surgery. Among the 28 patients in the CT group, four (14.3%) had leg lymphedema, one (3.6%) had grade 1 bowel obstruction, and two (7.1%)

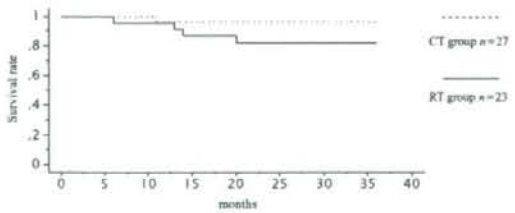

Figure 1 Three-year disease-free survival in 50 cases without multiple lymph node metastasis according to type of adjuvant therapy. CT, adjuvant chemotherapy; RT, pelvic radiotherapy.

Table 3 Rate of incidence of complications according to type of adjuvant therapy

Adjuvant therapy	No. patients	Data unavailable	Lymphedema	Bowel obstruction	Urinary disturbance
No treatment	55	7	8 (16.7%)[a]	5 (9.1%)[d]	12 (21.8%)[g]
Radiotherapy	42	4	12 (31.6%)[b]	13† (31.0%)[e]	16 (38.1%)[h]
Chemotherapy	28	0	3 (14.3%)[c]	1 (3.6%)[f]	2 (7.1%)[i]
Total	125	11	24 (19.2%)	19 (15.2%)	30 (24.0%)

Two cases required intestinal surgery. Lymphedema, a versus b: $P = 0.086$, b versus c: $P = 0.090$. Bowel obstruction, d versus e: $P = 0.007$, e versus f: $P = 0.0026$. Urinary disturbance, g versus h: $P = 0.089$, h versus i: $P = 0.0016$, g versus i: $P = 0.052$.

had urinary disturbance. The incidence of lymphedema tended to be higher in the RT group versus the other groups, but the difference was not statistically significant. Urinary disturbance was more frequent in the RT group versus the CT group ($P = 0.0016$) and no therapy group ($P = 0.089$). The incidence of bowel obstruction was significantly higher in the RT group than in the patients not receiving adjuvant treatment ($P = 0.007$) and in the CT group ($P = 0.0026$).

Discussion

A difference in the distribution of patients with lymph node metastasis across the treatment groups was found in this study, and we examined 50 patients without multiple LNM. We investigated the effect of adjuvant therapies after radical surgery in patients with intermediate risk factors for recurrence because it has been reported that multiple LNM has an effect on patient survival.[6–10]

Radical hysterectomy and radical RT for invasive cervical cancer are performed with the intent to cure the patient. However, these treatments can be associated with significant morbidity. Surgical complications are prevalent shortly after treatment, while complications of RT often occur years later. Combining these radical treatments increases the risk of complications compared to either treatment alone.[11–13] Regarding quality of life, radical hysterectomy has advantages over RT in terms of sexual function in young women with cervical cancer.[14] However, postoperative bladder dysfunction (neurogenic bladder) is a major disadvantage of radical hysterectomy. Recent attempts to avoid this complication with nerve-sparing surgical techniques have significantly decreased the risk of postoperative bladder dysfunction.[15–17] However, postoperative RT may extinguish the advantages of surgical therapy. As an alternative, some data support the clinical usefulness of CT as an adjuvant setting after radical hysterectomy.[18]

The most important prognostic factors of cervical cancer include parametrial extension of a cancer, positive surgical margins, and lymph node metastasis. Survival of node-positive patients is further affected by the site and number of positive nodes. An analysis in patients without multiple LNM revealed no significant difference in 3-year disease-free survival in CT versus RT. Although it has been suggested that adjuvant CT combined with radical hysterectomy and systematic lymphadenectomy has a survival benefit,[19] the role of adjuvant CT alone has not been extensively investigated.[18] In order to reduce the morbidity that may be caused by aggressive multimodality therapy, it seems important to conduct randomized trials to verify the effectiveness of this strategy.

The type of surgery is another important factor when considering adjuvant therapy. Japanese gynecologic oncologists use the Okabayashi operation and its modification, which correspond to type IV radical hysterectomy in the Piver-Rutledge classification. The extent of lymphadenectomy is also an important factor. Recent publications have shown that the number of lymph nodes removed is related to the survival of patients with various types of cancer, including breast, bladder, colon, and endometrial cancers, suggesting the therapeutic importance of systematic lymphadenectomy. Pieterse *et al.* found a significant relationship between the number of removed lymph nodes and the survival of cervical cancer patients with positive nodes.[20] Adjuvant RT is probably useful for eradicating tumor cells in unresected lymph nodes; we have evidence that postoperative pelvic radiation reduces the local recurrence of tumors at the cost of increased morbidity.

The failure pattern differs across adjuvant therapies. Adjuvant RT reduces local recurrence but not distant metastasis.[21] Adjuvant CT does not reduce local recurrence,[22] but reduces distant metastasis.[23] In the present study, there was a trend toward a difference between RT and CT in this regard, although not significant, because RT arm included more frequent cases with multiple LNM as a prognostic factor.

There was increased incidence of urinary disturbance and bowel obstruction in the RT group versus

the other groups. The incidence of postoperative interstitial complications with adjuvant RT was reported as 35.5% by Els *et al.*[24] and 48% by Bye *et al.*[25] Thus, radical hysterectomy followed by adjuvant RT results in a higher rate of bowel complications than CT.

Because of the non-randomized design of the study, we cannot draw any conclusion regarding the respective effects of the two adjuvant treatment modalities on overall survival. However, we can conclude that the effect of CT on disease-free survival was no worse than that of RT for patients without multiple LNM, and was associated with fewer bowel complications. Therefore, we believe that it is worth considering a prospective randomized trial of CT versus RT as an optional adjuvant therapy to patients with intermediate risk factors for recurrence.

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A phase II multicenter trial of concurrent chemoradiotherapy with weekly nedaplatin in advanced uterine cervical carcinoma: Tohoku Gynecologic Cancer Unit Study

YOSHIHITO YOKOYAMA[1], TADAO TAKANO[2], KENJI NAKAHARA[3], TADAHIRO SHOJI[4], HIROKAZU SATO[5], HIDEKAZU YAMADA[6], NOBUO YAEGASHI[2], KUNIHIRO OKAMURA[2], HIROHISA KURACHI[3], TORU SUGIYAMA[4], TOSHINOBU TANAKA[5], AKIRA SATO[6], TORU TASE[7] and HIDEKI MIZUNUMA[1]

[1]Department of Obstetrics and Gynecology, Hirosaki University School of Medicine, 5 Zaifu-cho, Hirosaki 036-8562; [2]Department of Obstetrics and Gynecology, Tohoku University School of Medicine, 1-1 Seiryo-cho, Aoba-ku, Sendai 980-8574, Sendai; [3]Department of Obstetrics and Gynecology, Yamagata University School of Medicine, 2-2-2 Iidanishi, Yamagata 990-9585; [4]Department of Obstetrics and Gynecology, Iwate Medical University School of Medicine, 19-1 Uchimaru, Morioka 020-8505; [5]Department of Obstetrics and Gynecology, Akita University School of Medicine, 1-1-1 Hondo, Akita 010-8543; [6]Department of Obstetrics and Gynecology, School of Medicine Fukushima Medical University, 1 Hikarigaoka, Fukushima 960-1295; [7]Department of Gynecology, Miyagi Cancer Center, 47-1 Aishima, Natori 981-1293, Japan

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Abstract. The purpose of this study was to evaluate the effectiveness and safety of concurrent chemoradiotherapy using weekly nedaplatin for the treatment of locally advanced squamous cell carcinoma of the uterine cervix. Nedaplatin at 30 mg/m^2 was administered weekly 6 times with a concurrent external beam and intracavity radiotherapy. External beam radiation was delivered with a fraction dose of 2 Gy per day for 5 days a week during a 5-week period and intracavitary brachytherapy, of which the fraction size is 6 Gy to point A, was given once a week for a total of 4 times using a remote after-loading system. Forty-five patients were enrolled in this trial between April 2003 and December 2006. Of the 45 patients, 40 (88.9%) completed the scheduled treatment and were evaluated for efficacy and safety. Of these, 4 were stage Ib2, 12 were stage IIb, 18 were stage IIIb and 6 were stage IVa. The age distribution ranged from 27 to 79 years with a median age of 58. The 40 patients achieved an objective response, 36 (90%) a complete response and 4 (10%) a partial response. At a median follow-up of 29 months (range, 8-52), the 3-year progression-free and overall survival were 58.7% (95% confidence interval, 42-75%) and 78.0% (95% confidence interval, 56-90.0%), respectively. Acute toxicities were transient and rendered non-lethal. Of the 45 patients enrolled for the trial, only 3 (6.7%) had grade 4 leukopenia and neutropenia, respectively. Grade 3 diarrhea and nausea/vomiting were observed in 2 (4.4%) and 1 (2.2%), respectively. These results indicate that weekly nedaplatin of 30 mg/m^2 with concurrent radiotherapy is an effective and well-tolerated regimen for advanced squamous cell carcinoma of the uterine cervix.

Correspondence to: Dr Yoshihito Yokoyama, Department of Obstetrics and Gynecology, Hirosaki University School of Medicine, 5 Zaifu-cho, Hirosaki 036-8562, Japan
E-mail: yokoyama@cc.hirosaki-u.ac.jp

Key words: advanced uterine cervical carcinoma, weekly nedaplatin, radiotherapy, phase II multicenter trial

Introduction

Although uterine cervical carcinomas have long been treated with surgery, radiation therapy alone, or a combination of the two, these treatments have a poor outcome, a fact resulting in a long-term debate on the need to combine them with chemotherapy. In the 1990s, numerous attempts were made to improve the prognosis for advanced uterine cervical carcinoma by concurrently using radiation therapy and chemotherapy. These attempts led to the announcement by the National Cancer Institute in the USA in February 1999 that the results of five randomized controlled trials demonstrated that the concurrent use of radiotherapy and chemotherapy (especially using cisplatin) was effective against advanced uterine cervical carcinoma and decreased the risk of death thereof by 30-50% (1-5). This concurrent chemoradiotherapy (CCRT) has become a current standard treatment in the USA for locally advanced uterine cervical carcinoma or pelvic extension of the carcinoma. However, data from these five clinical trials also showed several problems when this CCRT is employed in Japanese patients. The problems that need to be solved include that standard radiation therapy in Western countries is different to that in Japan. For example, most of the institutions

in Japan use the high-dose-rate remote after-loading system (HDR-RALS) for intracavitary brachytherapy as the standard treatment modality for uterine cervical carcinoma; whether or not the reported dose of cisplatin is appropriate for Japanese women, cisplatin should be administered weekly or at a longer interval than that, the use of cisplatin is appropriate for patients with advanced uterine cervical carcinoma because of their possibly reduced renal function and how effective platinum-containing drugs in the place of cisplatin will be in such patients, as well as how severe the late effects will be after the use of cisplatin.

Nedaplatin, an antineoplastic drug containing a platinum complex, was developed in order to provide superior antitumor effects to cisplatin and fewer adverse reactions such as the renal and gastrointestinal toxicities. Based on the results of a phase I clinical trial of nedaplatin, it was determined that the drug should be given as an intravenous infusion of 100 mg/m^2 at intervals of four weeks (6). A phase II clinical trial using this dosage regimen demonstrated a response rate of 46.3% (19/41 patients) in patients with uterine cervical carcinoma (7), which was superior to that obtained with cisplatin (35.9%, 14/39 patients). Regarding adverse drug reactions, although it was confirmed that the nephrotoxicity of nedaplatin was milder than that of cisplatin, certain patients developed grade 3 or 4 myelosuppression (33.6% for thrombocytopenia and 31.1% for leukopenia), which suggested that the use of nedaplatin needs extreme caution (7).

Since nedaplatin is associated with a higher response rate in uterine cervical carcinoma than in cisplatin, fewer gastrointestinal and renal disorders, and less fluid volume are needed. The replacement of cisplatin with nedaplatin may therefore be expected to provide a longer survival associated with a better QOL. Yoshinaga *et al* conducted a dose-finding study of CCRT using weekly nedaplatin in uterine cervical carcinoma and confirmed that the recommended dose is 35 mg/m^2 (8). Two phase I studies of radiation therapy combined with nedaplatin showed the optimal dose of weekly nedaplatin to be 30 mg/m^2, which could be administered with minor adverse drug reactions and with no delayed radiation therapy or postponed surgery plan (9,10).

At present, no evidence of CCRT in Japanese patients with advanced uterine cervical carcinoma exists. In our study, a multicenter trial of CCRT was planned using weekly nedaplatin in uterine cervical carcinoma to evaluate its tumor reduction effects, duration of response, survival time and occurrence of adverse events.

Patients and methods

Patients. Eligibility criteria were: 1) radiotherapy chosen as an initial treatment of the uterine cervical carcinoma and pathologically-proven squamous cell carcinoma, 2) target lesion measurable with RECIST, 3) age 80 years or younger, 4) the Eastern Cooperative Oncology Group (ECOG) performance status (PS) 0 or 1 and 5) the adequate function of the bone marrow, kidney and liver (leukocyte count >3000/mm^3, neutrophil count >1500/mm^3, platelet count >100,000/mm^3, total bilirubin <1.5 mg/dl, GOT (AST) or GPT (ALT) level less than twice the upper normal limit, and serum creatinine less than the upper normal limit). Acute toxicity was assessed according to the National Cancer Institute Common Toxicity Criteria, version 2.0, issued in 1999. Women were excluded from this study if they met any of the following criteria: 1) receiving prior radiotherapy or systemic chemotherapy for other diseases, 2) having other active carcinomas, 3) having serious complications or medical contraindications to chemotherapy and 4) showing disease outside the pelvic area or spread to the para-aortic lymph nodes. The study protocol was approved by the ethics committee of the Institutional Review Board of each participant institution. All of the patients gave their written informed consent before entering the trial. Registration of the patients was started in April 2003 and ended in December 2006.

Table I. Patient characteristics (n=45).

Age (years)	
Median	58
Range	27-29
FIGO stage	
Ib2	4
IIb	14
IIIb	21
IVa	6
Tumor size (cm)	
<4	6
4-6	17
6-8	19
>8	3
Pelvic lymphadenopathy	
Yes	20
No	25

Chemotherapy. Nedaplatin (30 mg/m^2) was dissolved in 500 ml saline solution and infused intravenously for >180 min. The first administration was performed on the starting day of the external beam radiation therapy. The regimen was repeated weekly for a total of six times. Nedaplatin infusion was completed 1 h before irradiation. Anti-emetics such as the 5-HT3 receptor antagonist were administered routinely before nedaplatin infusion. A granulocyte colony-stimulating factor was injected subcutaneously if patients had a neutrophil count of grade ≥3. Nedaplatin infusion was delayed if the peripheral neutrophil count was <1000/mm^3 or the peripheral platelet count was <75,000/mm^3.

Radiotherapy. External beam radiation was delivered with anteroposterior and posteroanterior opposed beams generated by an X-ray accelerator with an energy of 10 MV at a distance of 100 cm. Whole pelvis irradiation to a total dose of 50 Gy was delivered with a fraction dose of 2 Gy per day for 5 days a week during a 5-week period. After ~30 Gy was given, a central shield with a width of 4 cm at the midline was used and intracavitary brachytherapy was performed using a RALS with a Co-60 source. Intracavitary brachytherapy, of which

Table II. Response to the treatment.

	No. of patients	CR	PR	SD	PD
All stages	40	36 (90.0)	4 (10.0)	0 (0)	0 (0)
Stage Ib2-IIb	16	15 (93.8)	1 (6.20)	0 (0)	0 (0)
Stage IIIb-IVa	24	21 (87.5)	3 (12.5)	0 (0)	0 (0)

CR, complete response; PR, partial response; SD, stable disease and PD, progressive disease.

Table III. Site of disease progression.

Site of progression	No. of patients (%)
Local	10 (25)
Distant	4 (10)
No evidence of disease	26 (65)

Recurrences were classified as local if they were first detected in the pelvis, cervix or vagina and as distant if they were first detected outside the pelvis.

the fraction size is 6 Gy to point A, was given once a week for a total of 4 times. Radiotherapy was suspended when the peripheral neutrophil count was <500/mm^3 or the peripheral platelet count was <50,000/mm^3.

Patient evaluation. All the patients had weekly hematology and chemistry testing for safety as well as for the clinical evaluation of the disease during treatment. Patients were evaluated for response 4 weeks after finishing the treatment. Complete response was defined histologically and clinically as the disappearance of all gross lesions for 1 month after completion of the treatment. Partial response was defined as a >50% reduction of the tumor size for 1 month after completion of the treatment and an absence of new lesions. Stable disease was defined as the presence of the tumor with <50% reduction of the tumor size. Progressive disease was defined as the appearance of any new lesion during the treatment or a >25% increase in size of the local tumor.

Statistical analysis. The progression-free survival (PFS) and overall survival (OS) were calculated from the end of the treatment by the Kaplan-Meier method. A comparison between the survival curves was made using the log-rank test. A difference in the response rate between stages was analyzed using the Chi-square test. Statistical significance was set at $P<0.05$.

Results

Patient outcome. Forty-five patients were enrolled in this trial and were staged according to the International Federation of Gynecology and Obstetrics (FIGO) criteria. The number of patients with FIGO stage Ib2, IIb, IIIb and IVa was 4, 14, 21 and 6, respectively. Each of the 4 cases of FIGO stage Ib2 had a tumor diameter of 6 cm or larger. The age distribution ranged from 27 to 79 years with a median age of 58. The characteristics of these patients are listed in Table I. Forty patients out of the 45 (88.9%) completed the trial and were evaluated for the efficacy and safety of this treatment. Of the 40 patients, 33 were treated with external beam radiation and intracavitary brachytherapy. The remaining 7 patients received only external beam radiation, the dose ranging from 50 to 60 Gy. Out of the 45 enrolled patients, 5 abandoned the scheduled treatment. They voluntarily withdrew from the trial on the grounds of bone marrow suppression and anorexia. While all of the 45 enrolled in this trial were evaluated for toxicity, the 40 patients who completed the scheduled treatment were evaluated for response.

Patients who completed the planned treatment (36 out of the 40, 90%) showed a complete response and 4 out of those (10%) showed a partial response, achieving a highly successful treatment (100%) (Table II). No significant difference in the response rate between stages Ib2/IIb and IIIb/IVa (Table II) was found.

Fourteen patients relapsed or progressed after the treatment (Table III). Eleven complete responders relapsed (7 inside the radiation field, 3 in the para-aortic lymph nodes and 1 in the lungs). Of the 4 partial responders, 1 underwent a radical hysterectomy shortly after the completion of treatment and she survived without any evidence of disease for 43 months. The remaining 3 partial responders progressed locally despite palliative treatment.

At a median follow-up of 29 months (range, 8-52), the 3-year PFS and OS rates were 58.7% (95% confidence interval, 42-75%) and 78.0% (95% confidence interval, 56-90.0%), respectively (Fig. 1a and b). The 3-year PFS and OS rates were 68.7 and 86.5% for patients with stage Ib2 and IIb, respectively and 47.9 and 60.4% for those with stage IIIb and IVa, respectively. However, these differences were not significant (Table IV). The 3-year PFS and OS rates were significantly lower for the patients with pelvic lymph node swelling than for those without (74.5 vs. 39.2%, $P<0.01$ and 89.3 vs. 41.1%, $P<0.02$, respectively) (Table IV). No significant differences in the 3-year PFS and OS were seen between the tumor sizes (Table IV).

Toxicity. Acute toxicities were transient and rendered non-lethal. Of the 45 patients enrolled in the trial, only 3 (6.7%) had grade 4 leukopenia and neutropenia, respectively (Table V). No patient required blood or platelet transfusions. Grade 3