mechanism of carcinogenesis, because poorly differentiated carcinomas tend to be more frequent. As these patients tend to have a low BMI, this type of endometrial cancer cannot be explained by estrogen-dependent carcinogenesis. There were more cases of poorly differentiated early-onset endometrial cancer with lower BMI (thin patients), 5/8 cases (63%) with BMI < 20, so we suggest that early-onset endometrial carcinogenesis is related to genetic factors, except estrogen-dependent factors. However, the prognosis is not so poor in patients with a familial predisposition to cancer. It is interesting that frequent development of poorly differentiated tumors and favorable prognosis are reported in endometrial cancer associated with HNPCC.[25,30] In HNPCC, it is also reported that lymphocyte infiltration is significantly more frequent in the tumor tissue, suggesting that activation of cellular immunity may contribute to a favorable prognosis.[25,31-33] As some cases of early-onset endometrial cancer with familial predisposition to cancer may be caused by DNA mismatch repair gene mutations as in HNPCC, the favorable prognosis could result from a similar mechanism to that in HNPCC. Another report showed that infiltration of CD8+ lymphocytes into tumors was also involved with prognosis in endometrial cancer[34] and lymphocyte infiltration was frequently found in microsatellite instability (MSI)-positive tissues of endometrial cancer from our patients.[15]

Progesterone therapy, which is a potential therapy for fertility preservation in early-onset endometrial cancer, could be effective for estrogen-stimulated tumors, whereas its efficacy could be different for cases with a family history of cancer, because of different mechanisms of carcinogenesis. This could be one factor contributing to the limited efficacy of progesterone therapy against endometrial cancer. Currently, there are many reports concluding that fertility-preserving therapy is not appropriate in poorly differentiated endometrial carcinomas.[3,17] However, our study suggests that some cases of poorly differentiated carcinomas in early-onset endometrial cancer could show a favorable prognosis depending on the clinical background. This study suggested that the clinicopathological manifestations of early-onset endometrial cancer could be useful information in predicting the prognosis. Therefore, early-onset endometrial cancer should be divided into subgroups based on these clinicopathological manifestations, and therapeutic efficacy against early-onset endometrial cancer could be further improved by selecting the treatment according to the clinicopathological manifestations.

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Phase II trial of docetaxel in advanced or metastatic endometrial cancer: a Japanese Cooperative Study

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The purpose of this study was to determine whether docetaxel has antitumour activity in patients with advanced or recurrent endometrial carcinoma. Chemotherapy-naïve or previously treated patients (one regimen) with histopathologically documented endometrial carcinoma and Eastern Cooperative Oncology Group performance status $\leqslant 2$ entered the study. Docetaxel 70 mg m^{-2} was administered intravenously on day 1 of a 3-week cycle up to a maximum of six cycles. If patients responded well to docetaxel, additional cycles were administered until progressive disease or unacceptable toxicity occurred. Of 33 patients with a median age of 59 years (range, 39–74 years) who entered the study, 14 patients (42%) had received one prior chemotherapy regimen. In all, 32 patients were evaluable for efficacy, yielding an overall response rate of 31% (95% confidence interval, 16.1–50.0%); complete response and partial response (PR) were 3 and 28%, respectively. Of 13 pretreated patients, three (23%) had a PR. The median duration of response was 1.8 months. The median time to progression was 3.9 months. The predominant toxicity was grade 3–4 neutropenia, occurring in 94% of the patients, although febrile neutropenia arose in 9% of the patients. Oedema was mild and infrequent. Docetaxel has antitumour activity in patients with advanced or recurrent endometrial carcinoma, including those previously treated with chemotherapy; however, the effect was transient and accompanied by pronounced neutropenia in most patients.

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Clinical Studies

Most patients with endometrial cancer are diagnosed at an early stage when surgery alone may result in cure. However, the outcome for women with advanced stage or recurrent disease is poor and rarely curable. Both single-agent and combination regimens of chemotherapy have been studied in women with advanced endometrial carcinoma. Currently, no standard chemotherapy regimen for endometrial cancer exists, but single-agent doxorubicin is active, with responses observed in up to one-third of previously untreated patients (Moore *et al*, 1991). Other single agents with modest activity include cisplatin (Thigpen *et al*, 1984a, 1989) and carboplatin (van Wijk *et al*, 2003). Although the response rates with the combination doxorubicin–cisplatin appear to be higher than those achieved with either agent alone, there is no evidence that survival is any longer with combination therapy. In the Gynecologic Oncology Group (GOG) trial comparing doxorubicin alone with doxorubicin–cisplatin, the response rates and progression-free survival were better with the combination regimen (42 *vs* 25%, 5.7 *vs* 3.8 months, respectively), but overall survival (OS) had not significantly improved (Thigpen *et al*, 2004).

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The taxanes, paclitaxel and docetaxel, are potent chemotherapeutic agents that block tubulin depolymerisation, leading to the inhibition of microtubule dynamics, and have significant clinical efficacy for various solid tumours. Paclitaxel has been evaluated as an active agent for endometrial cancer (Ball *et al*, 1996; Lissoni *et al*, 1996; Lincoln *et al*, 2003). However, preclinical data show that docetaxel has increased potency and an improved therapeutic index compared with paclitaxel (Bissery *et al*, 1995), and its short 1-h infusion time offers a substantial clinical advantage over the prolonged infusion durations required with paclitaxel. Docetaxel and paclitaxel also have substantially different toxicity profiles. In particular, docetaxel has a significant lower incidence of neurotoxicity in comparison to paclitaxel (Hsu *et al*, 2004).

The present phase II trial was designed to evaluate the clinical efficacy and tolerability of docetaxel 70 mg m^{-2} in patients with advanced or recurrent endometrial cancer.

PATIENTS AND METHODS

Eligibility criteria

Eligible patients aged between 20 and 74 years, with a life expectancy in excess of 3 months, and Eastern Cooperative

Oncology Group (ECOG) Performance Status (PS) of 0–2 had histologically documented primary stage III, IV or recurrent endometrial carcinoma. Tumours were staged according to the International Federation of Gynecology and Obstetrics criteria. All patients had measurable disease according to the response evaluation criteria in solid tumours (RECIST) (Therasse *et al*, 2000). Measurable lesions defined unidimensionally were $\geqslant 20$ mm using conventional imaging, or $\geqslant 10$ mm with spiral computed tomographic scan. Patients were either chemotherapy-naive or had received one prior chemotherapy regimen for endometrial cancer, with 4 weeks between prior therapy and study treatment. Prior treatment with a taxane was not allowed. Adequate organ function was required for study entry: neutrophil count $\geqslant 2000\,\mu l^{-1}$, platelet count $\geqslant 100\,000\,\mu l^{-1}$, haemoglobin $\geqslant 9.0\,g\,dl^{-1}$, serum bilirubin level $\leqslant 1.5\,mg\,dl^{-1}$, normal hepatic function (asparate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) levels $\leqslant 2.5$ times upper limit of the institutional normal (ULN)), serum creatinine level $\leqslant 1.5\,mg\,dl^{-1}$, PaO_2 $\geqslant 60$ mmHg and normal electrocardiogram. Patients with any of the following conditions were excluded from the study: sarcoma component, active infection, severe heart disease, interstitial pneumonitis, past history of hypersensitivity, peripheral neuropathy, malignant or benign effusions requiring drainage, active brain metastasis, or active concomitant malignancy. All patients gave informed consent before entering this study, which was approved by the institutional review boards at all participating institutions.

Treatment schedule

Docetaxel $70\,mg\,m^{-2}$ was infused over a 1–2-h period. The treatment was repeated every 3 weeks unless there was documented disease progression or unacceptable toxicity. Prophylactic medications for nausea, vomiting or hypersensitivity reactions were given if these symptoms occurred. No routine premedication was given for hypersensitivity reactions and fluid retention during the first cycle of treatment. The patient's physician identified all hypersensitivity reactions and, if deemed necessary, the investigator administered premedication drugs.

Treatment was delayed for up to 3 weeks in the event of toxicity, but was restarted when the neutrophil count was $\geqslant 1500\,\mu l^{-1}$, platelet count $\geqslant 100\,000\,\mu l^{-1}$, AST/ALT/ALP levels $\leqslant 2.5$ times ULN, and neuropathy or oedema $\leqslant$ grade 1. Docetaxel dosage was reduced by $10\,mg\,m^{-2}$ if febrile neutropenia occurred, if there was bleeding with grade 3–4 thrombocytopenia requiring a platelet transfusion, or if a patient experienced any grade 3–4 nonhaematologic toxicities except nausea, vomiting, anorexia, fatigue, alopecia or hypersensitivity.

Response and toxicity evaluation

The tumour response was assessed according to the standard RECIST criteria (Therasse *et al*, 2000). Target lesions included all measurable lesions up to a maximum of five lesions per organ and 10 lesions in total. Complete response (CR) was defined as the complete disappearance of all target and nontarget lesions, with no development of new disease. Partial response (PR) was defined as a reduction by $\geqslant 30\%$ in the sum of the longest diameter of target lesions. Complete response or PRss were confirmed by repeat assessments performed no less than 4 weeks after the criteria for response were first met. Progressive disease (PD) was defined as an increase by $\geqslant 20\%$ in the sum of the longest diameter of all target lesions, or the appearance of one or more new lesions and/or unequivocal progression of existing, nontarget lesions. Stable disease (SD) was defined as neither sufficient lesion shrinkage to qualify for a PR, nor sufficient increase to qualify for PD. Best response was defined as the most CR achieved by a patient (thus, each patient had a single best response: CR, PR, SD or PD), and the date of best response was the date it was first detected. Time to progression (TTP) was defined as the time from the first medication to the date of a PD event or death (due to endometrial cancer or study drugs). All tumours were radiographically assessed for response every 6 weeks. An independent response review committee (IRRC) evaluated all tumour responses after the investigators had completed their judgement.

Toxicities were evaluated with respect to incidence and severity using National Cancer Institute common toxicity criteria (NCI-CTC, version 2.0) (Trotti *et al*, 2000).

Statistical consideration

Assuming a response rate of 20%, the study was designed with 80% power such that the lower limit of the 95% confidence interval (CI) for the estimate of the response rate was greater than 0.05. A sample size of 32 evaluable patients was required.

The primary end point was overall tumour response (determined by the IRRC) with the corresponding 95% CI using the exact binominal method for the evaluable population. The secondary end point of this study was safety. The Kaplan–Meier (KM) method was used to determine the TTP and median survival time (MST) in the evaluable population.

RESULTS

Patient characteristics

A total of 33 patients were enrolled on the study from April 2001 to October 2003 and one patient was unevaluable as a result of having received prior treatment with paclitaxel and doxorubicin–platinum regimens. The median age of the intent to treat (ITT) population ($n = 33$) was 59 years (range 39–74) and 70% patients had ECOG PS 0 (Table 1). Several patients had unfavourable histologic characteristics: adenosquamous features (three) and uterine papillary serous cancers (two). Most patients (88%) had undergone total abdominal hysterectomy and bilateral salpingo-oophorectomy, and one-third of patients had prior radiotherapy. Of those patients who had received prior chemotherapy ($n = 14$), 10 had received combination doxorubicin–platinum in combination, three had received platinum alone and one had received oral fluorouracil. All 33 patients were evaluated for toxicity and survival, while 32 patients were evaluated for response and TTP.

Treatment delivery

Overall, 32 patients received a total of 133 cycles of docetaxel and the median number of cycles of docetaxel was four (range, 1–13). Five patients (15%) experienced dose reductions for the following reasons: two patients experienced febrile neutropenia (in one patient this occurred twice) and three patients had grade 3 nonhaematologic toxicities: diarrhoea (occurred twice in one patient), hyperglycaemia, hyperkalaemia and supraventricular tachycardia.

Response

Table 2 presents the assessment of response to treatment. Two patients, one who was chemotherapy-naïve and the other who had received prior therapy, were not assessable for response because they had received only one cycle of treatment. Before evaluation by the IRRC, primary physicians had reported two CRs and nine PRs. The IRRC judged one CR as a PR, two PR as SD and one SD as a PR. Therefore, the overall response rate for 10 of 32 patients was 31% (95% CI, 16.1–50.0%). Of 13 patients who had prior chemotherapy, three (23%) achieved a PR: two had received doxorubicin–platinum and one platinum alone. The histologic analysis revealed responses among the following tumour types:

endometrioid adenocarcinoma (6 of 25 patients), squamous differentiated adenocarcinoma (1 of 3), papillary serous (2 of 2) and undifferentiated cancer (1 of 1). The median time for the onset of effect was 2.0 months (range, 0.7–4.5) and the median duration of response was 1.8 months (range, 0.9–4.6). The median follow-up time was 17.6 months (range, 1.7–36.3) and median TTP was 3.9 months (95% CI, 1.5–10.2 months) (Figure 1). Median survival time was 17.8 months (95% CI, 7.4–22.0 months).

Safety and toxicity

In all, 33 patients were assessable for toxicity (Table 3). Also, 31 (94%) patients experienced grade 3 or 4 neutropenia, and three (9%) developed febrile neutropenia. Nonhaematologic toxicities included grade 3 anorexia and vomiting experienced by some patients (18 and 9%, respectively). One patient experienced grade 3 peripheral neuropathy (sensory and motor) after five treatment cycles. Three patients terminated the study as a consequence of the following toxicities: infection with *Mycobacterium avium* complex (one), grade 4 hypersensitivity reaction despite premedication with dexamethasone (one) and grade 3 oedema with pleural effusion after six treatment cycles (one). All three patients recovered after receiving recommended medical treatment. There were no treatment-related deaths.

Table 1 Patient characteristics

Characteristic	No. of patients ($n = 33$)
Age, years	
Median	59
Range	39–74
ECOG performance status	
0	23
1	9
2	1
Disease status	
Stage III, IV	9
Recurrent	24
Histology	
Endometrioid	26
Adenocarcinoma with squamous differentiated	3
Papillary serous	2
Adenocarcinoma, unspecified	1
Undifferentiated	1
Tumour grade	
1	11
2	11
3	6
Unknown	5
Prior treatment	
Surgery	29
Radiotherapy	9
Hormonal therapy	5
Prior chemotherapy	
None	19
Doxorubicin and platinum	9
Platinum alone	3
Others	2

ECOG = Eastern Cooperative Oncology Group.

DISCUSSION

At initial diagnosis, only a small percentage of endometrial cancer patients have recurrent or advanced disease with distant metastases, and therefore a multicentre trial is essential for the accrual of patients. This multicentre phase II trial, although relatively small in sample size, clearly demonstrated that docetaxel is active in the treatment of endometrial cancer. Toxicity was manageable and predominantly haematologic.

Taxanes have shown activity in this setting previously, with paclitaxel demonstrating overall response rates of 27–37% when used as a single agent in endometrial cancer (Ball *et al*, 1996; Lissoni *et al*, 1996; Lincoln *et al*, 2003). Combination chemotherapy with paclitaxel and carboplatin or cisplatin has resulted in response rates of 50–56% (Dimopoulos *et al*, 2000; Hoskins *et al*,

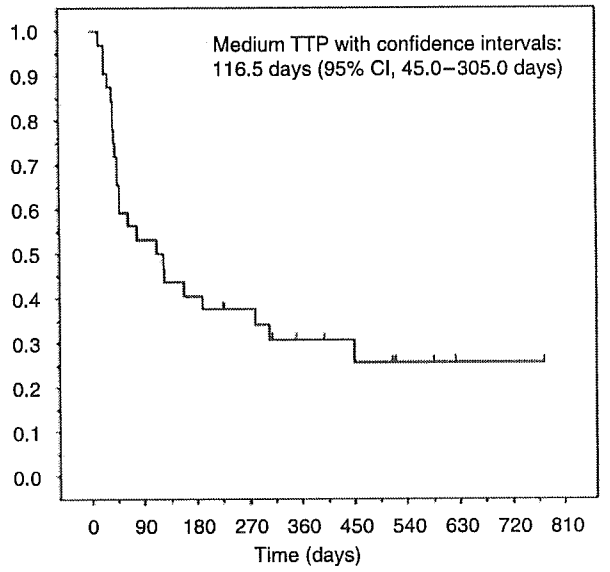

Figure 1 KM curve of estimated TTP.

Table 2 Best response (RECIST criteria) to docetaxel

	Prior chemotherapy ($n = 13$)		No prior chemotherapy ($n = 19$)		Total ($n = 32$)	
Response	**No. of patients**	**%**	**No. of patients**	**%**	**No. of patients**	**%**
Complete response	0	0	1	5	1	3
Partial response	3	23	6	32	9	28
Stable disease	4	31	5	26	9	28
Progressive disease	5	38	6	32	11	34
Not assessable	1	8	1	5	2	6
ORR (95% CI)		23 (5.0–53.8)		37 (16.3–61.6)		31 (16.1–50.0)

ORR = overall response rate; CI = confidence interval.

Table 3 Adverse effects

	NCI-CTC grade ($n=33$)									
	1		2		3		4		3–4	
Toxicities	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Neutrophils	1	3	0	0	10	30	21	64	31	94
Haemoglobin	11	33	11	33	1	3	1	3	2	6
Lymphopenia	1	3	14	42	11	33	—	11	33	
Platelets	6	18	1	3	0	0	0	0	0	0
Alopecia	5	15	26	79	—	—	—			
Fatigue	13	39	7	21	3	9	0	0	3	9
Anorexia	12	36	5	15	6	18	0	0	6	18
Nausea	16	49	6	18	2	6	—	2	6	
Vomiting	7	21	3	9	3	9	0	0	3	9
Diarrhoea	14	42	3	9	3	9	0	0	3	9
Constipation	2	6	10	30	4	12	0	0	4	12
Stomatitis	3	9	5	15	1	3	0	0	1	3
Febrile neutropenia	—	—	3	9	0	0	3	9		
Infection	0	0	3	9	0	0	0	0	0	0
Oedema	7	21	3	9	1	3	0	0	1	3
Neuropathy-motor	1	3	0	0	1	3	0	0	1	3
Neuropathy-sensory	9	27	2	6	1	3	0	0	1	3
Supraventricular arrhythmia	0	0	0	0	1	3	0	0	1	3
Allergic reaction	3	9	0	0	0	0	1	3	1	3
Rash/desquamation	6	18	5	15	1	3	0	0	1	3
Injection site reaction	5	15	2	6	0	0	0	0	0	0
Nail changes	4	12	0	0	—	—	—			
AST	9	27	3	9	0	0	0	0	0	0
ALT	8	24	2	6	0	0	0	0	0	0
Hypokalaemia	0	0	—	3	9	0	0	3	9	

NCI-CTC = National Cancer Institute common toxicity criteria; AST = asparate aminotransferase; ALT = alanine aminotransferase. Present NCI-CTC grade 3–4 in >5% patients and breakdown if possible by whether patient had prior chemotherapy.

2001; Scudder *et al*, 2005). However, a GOG randomised trial of women with advanced or recurrent endometrial carcinoma, in which the combination paclitaxel–doxorubicin was compared with doxorubicin–cisplatin, showed that the paclitaxel arm did not result in an improved outcome (Fleming *et al*, 2000). A subsequent GOG study, in which the combination paclitaxel, doxorubicin and cisplatin (TAP) with G-CSF was compared with doxorubicin–cisplatin, showed that the TAP arm yielded a better response (57 *vs* 34%; $P<0.01$), progression-free survival (median, 8.3 *vs* 5.3 months; $P<0.01$) and OS (median, 15.3 *vs* 12.3 months; $P=0.037$) than the control arm. However, more grade 3 neuropathy (12 *vs* 1%) and congestive heart failure were observed with TAP than with doxorubicin–cisplatin (Fleming *et al*, 2004). In light of this imbalance between efficacy and toxicity, TAP has not been accepted as the standard chemotherapy regimen in routine clinical practice.

Docetaxel has a toxicity profile that is different from paclitaxel. In particular, neurotoxicity occurs at a low incidence with docetaxel. In our study, only one patient developed grade 3 neuropathy-sensory and recovered in several weeks. While fluid retention is a distinctive toxicity of docetaxel, this can be prevented using premedication (Piccart *et al*, 1997); in our trial, one patient developed pleural effusion since the routine premedication with corticosteroids was not applied.

Several studies have reported on second-line chemotherapy for endometrial cancer. Two phase II trials of second-line paclitaxel report response rates of 27% (12 out of 44) and 37% (7 out of 19) (Lissoni *et al*, 1996; Lincoln *et al*, 2003). An older report describes a 30% response rate to second-line high-dose cisplatin (3 mg kg^{-1}) among 13 patients (Deppe *et al*, 1980). With the exception of these studies, response rates to second-line chemotherapy are uniformly less than 20% and most are less than 10% (Slayton *et al*, 1982, 1988; Stehman *et al*, 1983; Thigpen *et al*, 1984b, 1986; Homesley *et al*, 1986; Asbury *et al*, 1990; Muss *et al*, 1991, 1993; Sutton *et al*, 1994; Rose *et al*, 1996; Muggia *et al*, 2002). In our study, 23% of pretreated patients (3 out of 13) had a PR to docetaxel, suggesting that it too is active as second-line therapy.

In conclusion, this multicentre phase II trial shows that docetaxel is active in the treatment of chemotherapy-naïve and chemotherapy pretreated patients with advanced or recurrent endometrial cancer and possesses a manageable toxicity profile; however, the effect was transient and accompanied by pronounced neutropenia in most patients. The exploration of the efficacy of docetaxel combinations in phase III studies for the treatment of endometrial cancer is of great interest and will be initiated.

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Appendix

The following institutions (with principal investigators) participated in this study: Sapporo Medical University, Sapporo, Satoru Sagae; Niigata University, Niigata, Kenichi Tanaka; Tochigi National Hospital, Utsunomiya, Masaaki Kikuchi; National hospital Organization Saitama Hospital, Wako, Mikio Mikami; National Cancer Center Hospital, Tokyo, Noriyuki Katsumata; Tokyo Women's Medical University, Tokyo, Hiroaki Ohta; School of medicine, Keio University, Tokyo, Daisuke Aoki; St. Marianna University School of Medicine, Kawasaki, Kazushige Kiguchi; Kitasato University, Sagamihara, Hiroyuki Kuramoto; Nagoya City University, Nagoya, Atsushi Arakawa; Fujita Health University, Toyoake, Yasuhiro Udagawa; Aichi Cancer Center Hospital, Nagoya, Kazuo Kuzuya; Osaka Medical College, Takatsuki, Ken Ueki; Kinki University, Osakasayama, Hiroshi Hoshiai; Hyogo Medical Center for Adults, Akashi, Ryuichiro Nishimura; Wakayama Medical University, Wakayama, Katsuji Kokawa; Okayama University, Okayama, Junichi Kodama; Okayama Red Cross General Hospital, Okayama, Kohei Ejiri; Kawasaki Medical School, Kurashiki, Ichiro Kohno; National Kyushu Cancer Center,

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Sensitivity to cisplatin determined by the histoculture drug response assay and clinical response of endometrial cancer

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Abstract. Kanasugi M, Aoki D, Suzuki N, Susumu N, Nakata S, Horiuchi M, Udagawa Y, Nozawa S. Sensitivity to cisplatin determined by the histoculture drug response assay and clinical response of endometrial cancer. *Int J Gynecol Cancer* 2006;**16**:409–415.

This study investigated the value of the *in vitro* histoculture drug response assay (HDRA) for predicting the efficacy of chemotherapy in patients with endometrial cancer. Specimens were obtained from 115 patients with endometrial cancer treated at Keio University Hospital between 1994 and 2002. Tumor fragments were cultured on collagen sponge gel with cisplatin for 7 days, and cell viability was assessed. The cutoff value of the 50% inhibitory concentration of cisplatin was set at 23 μg/mL. Sensitivity of stage III or IV disease to chemotherapy was investigated, and differences of 5-year progression-free survival between patients with sensitive and resistant tumors were evaluated by the Kaplan–Meier method. Tumors were evaluable in 93.0% of patients (107/115). Among 38 patients in stages III or IV, 23 received chemotherapy containing cisplatin. Seven sensitive tumors did not recur, while recurrence/progression occurred within 6 months in 8/16 patients with tumors showing low sensitivity. Among stages III and IV patients, there was a significant difference of 5-year progression-free survival ($P < 0.05$) between those with tumors showing high or low sensitivity. Accordingly, the HDRA may predict the efficacy of chemotherapy for endometrial cancer.

KEYWORDS: cisplatin, endometrial cancer, histoculture drug response assay.

Both irradiation and chemotherapy have become mainstream postoperative treatments for endometrial cancer and are often performed when patients have various clinicopathologic risk factors[1], although there is still some controversy as to which modality is more appropriate. At the 2003 Annual Meeting of the American Society of Clinical Oncology, the results of the GOG122 study were reported. This phase III randomized clinical study compared par-abdominal irradiation with doxorubicin plus cisplatin chemotherapy (AP therapy) in patients who had progressive endometrial cancer and demonstrated significantly longer progression-free survival and overall survival after AP therapy in comparison with radiotherapy. These findings suggested that chemotherapy could become the standard form of postoperative therapy for endometrial cancer[2].

Burke reported that the response rate to cyclophosphamide, doxorubicin, and cisplatin (CAP) therapy was 45% in 87 patients with progressive or recurrent endometrial cancer[3], while Thigpen *et al.* found a response rate of 45% to AP therapy[4]. At present, both CAP and AP therapy are widely used as first-line, multiple-agent adjuvant chemotherapy regimens for high-risk patients with endometrial cancer. However, many patients show an inadequate response to chemotherapy. Thus, a test that could assess the activity of anticancer agents against each patient's tumor might improve the response rate and would also help to avoid adverse reactions caused by the administration of ineffective drugs.

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Several *in vitro* drug sensitivity tests have been developed for solid tumors. In 1966, Kondo *et al.* reported the succinic dehydrogenase inhibitor method. In this method, a tumor cell suspension is mixed with an anticancer agent, and the viable cell count is determined by the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2 H-tetrazolium bromide (MTT) assay[5,6]. Because the evaluation rate is relatively high (80%) and the true negative rate is 91–97%, this method is useful for excluding ineffective drugs and also has the advantage of being fairly rapid. However, contamination by fibroblasts can interfere with interpretation of the results, and the assay is not useful for assessing the sensitivity of time-dependent drugs because of difficulty in maintaining cultures for long enough. Furthermore, the true positive rate is only 60–70%, so the succinic dehydrogenase inhibitor method is not particularly helpful for selecting effective drugs.

In 1977, Hamburger and Salmon developed the human tumor clonogenic assay, which evaluates drug activity based on the number of colonies formed during culture on soft agar, and this method has been reported to be effective for predicting the tumor response[7]. Von Hoff *et al.* reported that the true negative and true positive rates of the assay were 91% and 69%, respectively[8], so it may be useful for excluding ineffective drugs as in the succinic dehydrogenase inhibitor method but would not be so useful for selecting effective drugs. Also, the tumor evaluation rate is only 30–70%, and the clonogenic assay has the disadvantages of being complicated and requiring 2–3 weeks for evaluation.

In 1989, Hoffman *et al.* developed the histoculture drug response assay (HDRA), in which tumor tissue blocks were cultured and sensitivity was evaluated from ^{3}H-thymidine uptake; they reported that evaluation was possible for more than 90% of tumors[9]. As a modification of this method, Furukawa *et al.*[10] transplanted various tumors (including stomach cancer, colon cancer, breast cancer, lung cancer, hepatocellular carcinoma, and neuroblastoma) into nude mice and assessed cell viability by the MTT assay. They obtained true positive, true negative, and accuracy rates of 89.8%, 90%, and 90%, respectively, and concluded that this method was useful for predicting the efficacy of anticancer agents[10]. We have also used the HDRA to assess the response of ovarian cancer to cisplatin, employing the MTT assay to determine cell viability because it is simpler than measuring ^{3}H-thymidine uptake. We achieved true positive, true negative, and accuracy rates of 88%, 86%, and 87%, respectively, and the tumor evaluation rate was also extremely high (97%), suggesting that the HDRA was a promising *in vitro* sensitivity test for predicting the response to chemotherapy[11].

With respect to the *in vitro* sensitivity testing of endometrial cancer, Ngyuyen reported on evaluation of cultured endometrial cells by the ATP chemosensitivity assay[12], while Hiramatsu *et al.* assessed cultured endometrial cancer cells by crystal violet staining[13]. However, both of these studies employed cultured tumor cells, and there have been no reports published regarding the relationship between *in vitro* sensitivity testing and the clinical response of endometrial cancer.

Accordingly, the present study was performed to assess the possible clinical application of the HDRA for predicting the efficacy of chemotherapy in endometrial cancer patients.

Materials and methods

Patients

The subjects of this study were 115 patients with endometrial cancer who underwent surgery between June 1994 and February 2002 at Keio University Hospital. Our institutional ethics committee approved the study protocol, and informed consent was obtained from all of the patients before the collection of tumor specimens. The FIGO surgical stage[14] was I, II, III, and IV in 64, 12, 29, and 10 patients, respectively. Among the 115 patients, 106 had endometrioid adenocarcinoma, which was well-differentiated adenocarcinoma (G1), moderately differentiated adenocarcinoma (G2), and poorly differentiated adenocarcinoma (G3) in 63, 29, and 14 patients, respectively. The other nine patients had serous adenocarcinoma ($n = 1$), clear-cell adenocarcinoma ($n = 4$), mucinous adenocarcinoma ($n = 2$), and carcinosarcoma ($n = 2$).

After surgery, 39 patients received chemotherapy, 16 patients received irradiation, and 60 patients had no further therapy.

Among the patients who received adjuvant chemotherapy containing cisplatin and underwent the HDRA, eight had measurable lesions (Table 1).

Methods

Collagen gel sponge (Gelfoam, Pharmacia & Upjohn, Kalamazoo, MI) was cut into approximately 1-cm^3 cubes and placed into the wells of a 24-well plate (Sumilon, Sumitomo Bakelite, Tokyo, Japan). As the culture medium, F-12 medium (Gibco Laboratories, Grand Island, NY) was prepared with 20% fetal bovine serum (Mitsubishi Chemical, Tokyo, Japan) and 80 μg/mL kanamycin (Meiji Seika, Tokyo, Japan),

Table 1. Cisplatin sensitivity in the HDRA and clinical response of patients with measurable lesions[a]

Case no.	IC_{50} (μg/mL)	Clinical response	Sites of measurable disease	Correlation	Chemotherapy
1	7.7	PR	Liver, parametrium	TP	CAP
2	16.9	CR	Lung	TP	CAP
3	21.5	PR	Liver	TP	CAP
4	23.4	PD	Local tumor, peritoneum	TN	CAP
5	33.4	PD	Peritoneum, lymph node	TN	CBDCA
6	34.7	NC	Parametrium	TN	TXL + CBDCA
7	115.0	PR	Lymph node	FP	CAP
8	151.8	PD	Lung	TN	CAP

[a]The response to treatment was classified as complete remission (CR), partial remission (PR), no change (NC), and progressive disease (PD). Correspondence with the HDRA was classified as true positive (TP), true negative (TN), or false positive (FP). The chemotherapy regimens included CAP (cyclophosphamide + doxorubicin + cisplatin), CBDCA (carboplatin), and TXL (paclitaxel) + CBDCA.

and 1 mL of this medium was added to each well. The concentration of cisplatin (Nippon Kayaku, Tokyo, Japan) in the medium was set at 6.25, 12.5, 25, 50, and 100 μg/mL, and four wells were used to test each concentration. Wells without cisplatin were also employed as a control.

Surgical specimens were collected aseptically and cut into approximately 1-mm^3 cubes with scissors. Then a tissue fragment was placed on the collagen gel in each well of the 24-well plate, and the specimens were incubated for 7 days at 37°C under an atmosphere of 5% CO_2. After incubation, the viable cell count was determined by the MTT assay. In brief, 100 μL of MTT (Sigma Chemical, St. Louis, MO) dissolved in phosphate buffered saline (5 mg/mL) containing 100 mM succinic acid (Wako Pure Chemical Industries, Osaka, Japan) was aseptically added dropwise to each well, and then incubation was continued for 4 h at 37°C under an atmosphere of 5% CO_2. Next, the tumor tissue specimens were transferred to a new 24-well plate, and MTT–formazan was extracted with dimethyl sulfoxide (1 mL/well) (Wako Pure Chemical Industries, Japan), after which 100 μL of the extract was transferred to each well of a 96-well microplate (Sumilon, Sumitomo Bakelite), and the absorbance at 540 nm was determined using a microplate reader (Model 450, Bio-rad Laboratories, Herculus, CA). The wet weight of each piece of tumor tissue was measured after extraction of MTT-formazan, and the absorbance per 1 g of tumor tissue was calculated. Then, the growth inhibition rate was calculated by the following equation, which compares the difference between treated and untreated cultures: Growth inhibition rate (%) = (1 − [absorbance per gram of tumor treated with cisplatin/absorbance per gram of tumor treated without cisplatin]) × 100.

A dose-response curve was plotted using the mean growth inhibition rate determined by testing four wells at each concentration, and the concentration that inhibited tumor growth by 50% (inhibitory concentration [IC_{50}] value) was calculated. Ideally, the cutoff IC_{50} value for evaluating sensitivity would be set by comparing the assay results with the actual clinical response, but there were only a few patients with measurable lesions in the present study. Therefore, the cumulative efficacy rate curve was plotted from the IC_{50} values obtained, and the cutoff IC_{50} value was set at 23 μg/mL based on the response rate (21%) of endometrial cancer to cisplatin monotherapy reported by Edmonson *et al.*[15] (Fig. 1). Tumor sensitivity was classified as high when the IC_{50} value was below the cutoff value, while it was classified as low when the IC_{50} value was above the cutoff value.

The response of the patients with measurable lesions was evaluated according to WHO criteria[16]

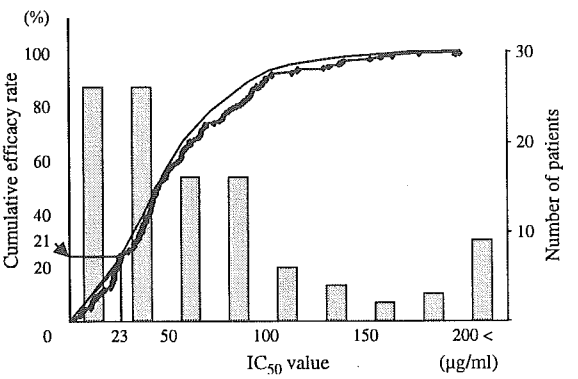

Figure 1. Cumulative efficacy of cisplatin-based therapy for endometrial cancer and histogram of the IC_{50} values of cisplatin. Because the clinical response rate of endometrial cancer to cisplatin alone was 21%, the intersection of this value with the cumulative efficacy curve was specified as the cutoff IC_{50} value for the HDRA (ie, 23 μg/mL). The HDRA was performed using specimens from 115 patients with endometrial cancer, and evaluation was possible in 106 patients. The mean IC_{50} value was 176.8 μg/mL.

based on computed tomography of the thorax, abdomen, and pelvic cavity. To assess the relationship between the clinical response and tumor sensitivity in the HDRA, tumors showing complete remission or partial remission (PR) were classified as "responsive," while those showing no change or progressive disease were classified as "resistant."

Thirty-three of the 39 patients receiving chemotherapy had endometrioid adenocarcinoma. In these patients, Welch's *t* test was used to assess the differences in the sensitivity to cisplatin between G1, G2, and G3 disease.

If the tumor was found to have a high sensitivity by the HDRA and showed a response to chemotherapy, this was classified as a true positive result, while tumors judged to have a low sensitivity that showed resistance to chemotherapy were classified as true negative. Similarly, tumors with a high sensitivity in the HDRA that showed resistance to chemotherapy were classified as false positive, while lesions with a low HDRA sensitivity that responded to chemotherapy were classified as false negative. The accuracy rate was calculated from the following equation: Accuracy rate = (Number of true positive tumors + Number of true negative tumors)/Total number of evaluable tumors.

When patients receiving chemotherapy had measurable lesions, the response of these lesions was considered to directly reflect their sensitivity to drug therapy, and the tumor response was compared with the evaluation of sensitivity based on IC_{50} values. Since there were only a few patients with measurable lesions after surgery, sensitivity to chemotherapy was evaluated in most of the subjects by using recurrence/progression versus nonrecurrence as an indicator of efficacy. However, the prognosis of stage I or II endometrial cancer is relatively good[17] and the recurrence rate is very low, making it difficult to evaluate the relationship between tumor sensitivity and recurrence/progression. Therefore, this relationship was only assessed in patients with stage III or IV disease. The Kaplan–Meier method was used to investigate the significance of differences in 5-year progression-free survival between high and low sensitivity tumors, while the Cox–Mantel test was used to assess differences in the prognosis.

Results

Tumor evaluation rate

Evaluation was possible in 107 of the 115 patients tested by the HDRA, so the tumor evaluation rate was 93.0%. Evaluation was impossible because of poor color development during the MTT assay in seven patients and because of contamination in one patient.

Relationship between the HDRA and the clinical response

Figure 1 shows a histogram of the IC_{50} values. The mean IC_{50} value was 176.8 μg/mL. When the cutoff IC_{50} value was set at 23 μg/mL, 22 out of 106 evaluable patients (20.4% of all patients) were classified into the high-sensitivity group. Measurable lesions were present in 8 out of 115 patients (Table 1). Among them, three tumors showed a high sensitivity in the HDRA, and the clinical response was complete remission in one case and PR in two cases. On the other hand, five tumors were accessed to show a low sensitivity, with the actual response being PR, no change, and progressive disease in one, one, and three cases, respectively. Based on these results, the true positive rate, true negative rate, and accuracy rate were 100%, 80%, and 87.5%, respectively (Table 2).

Relationship between the HDRA and recurrence/progression of stage III or IV disease

Among the 115 patients, 38 were in stage III or IV, and 23 of them received chemotherapy containing cisplatin. Of them, 7 and 16 had tumors that showed high and low sensitivity in the HDRA, respectively. There was no recurrence of the highly sensitive tumors, while recurrence/progression occurred in 8 of the 16 patients with tumors showing a low sensitivity. Recurrence or progression occurred within 6 months after the start of treatment in seven of those eight patients (Table 3). When the clinical response to cisplatin and tumor sensitivity in the HDRA were compared based on the presence or absence of recurrence/progression, the true positive rate, true negative rate, and accuracy rate were 100%, 50%, and 65.2%, respectively (Table 4).

Table 2. Relationship between the HDRA and clinical response in patients with measurable lesions[a]

	Clinical response		
Sensitivity	Responsive	Resistant	Total
High	3	0	3
Low	1	4	5
Total	4	4	8

[a]The optimal cutoff value of cisplatin was set at 23 μg/mL based on the reported response rate. True positive rate = 3/3 = 100%, true negative rate = 4/5 = 80%, accuracy rate = 7/8 = 87.5%.

Table 3. Sensitivity according to the HRDA and recurrence/progression in stage III or IV patients

Case no.	Stage	IC_{50} (μg/mL)	Recurrence	Progression-free interval (months)	Chemotherapy	Observation period (months)
1	IIIA	1.5	−		CAP	20
2	IIIC	2.7	−		CAP	26
3	IVB	4.4	−		CAP	94
4	IVB	7.7	−		CAP	7
5	IVA	12.0	−		CAP	89
6	IIIC	14.0	−		CAP	73
7	IVA	21.5	−		CAP	5
8	IVB	23.4	+	2	CAP	7
9	IVA	23.6	−		CAP	14
10	IIIA	29.3	−		CAP	60
11	IIIC	33.4	+	5	CBDCA	5
12	IIIC	34.7	+	2	TXL + CBDCA	18
13	IIIA	35.0	−		CAP	9
14	IIIA	37.2	−		CAP	87
15	IIIC	45.6	−		CAP	8
16	IIIC	77.7	+	5	CAP	12
17	IIIC	105.8	−		CAP	17
18	IIIC	112.0	−		CAP	27
19	IIIC	115.0	+	2	CAP	4
20	IIIB	151.8	+	5	CAP	26
21	IIIC	200<	+	7	CAP	82
22	IVB	200<	−		CAP	14
23	IIIC	200<	+	4	CAP	5

CAP, cyclophosphamide + doxorubicin + cisplatin; CBDCA, carboplatin; TXL, paclitaxel + CBDCA.

Prognosis of stage III or IV disease

The 5-year progression-free survival rate was determined for 23 patients with stage III or IV disease. It was 100% for 7 patients with tumors that showed a high sensitivity in the HDRA and 42.9% for 16 patients whose tumors showed a low sensitivity, and there was a significant difference between these two groups ($P < 0.05$) (Fig. 2).

Sensitivity of endometrioid adenocarcinoma with differing histology

There were no significant differences in the cisplatin sensitivity of endometrioid adenocarcinoma between the different levels of histologic differentiation (Fig. 3).

Table 4. Relationship between sensitivity and recurrence/progression in stage III or IV patients[a]

	Recurrence/progression		
Sensitivity	Positive	Negative	Total
Positive	7	0	7
Negative	8	8	16
Total	15	8	23

[a]True positive rate = 7/7 = 100%, true negative rate = 8/16 = 50%, accuracy rate = 15/23 = 65.2%.

Discussion

In the present study, the tumor evaluation rate of endometrial cancer was 93% by the HDRA, which was clearly higher than with the succinic dehydrogenase inhibitor method and the human tumor clonogenic assay. This was similar to the evaluation rate for gastric cancer and large-bowel cancer (96.3%) reported by Furukawa *et al.*[18], and the rate for ovarian cancer (97%) reported by Ohie *et al.*[11]. Accordingly, our results suggest that the HDRA is also applicable to

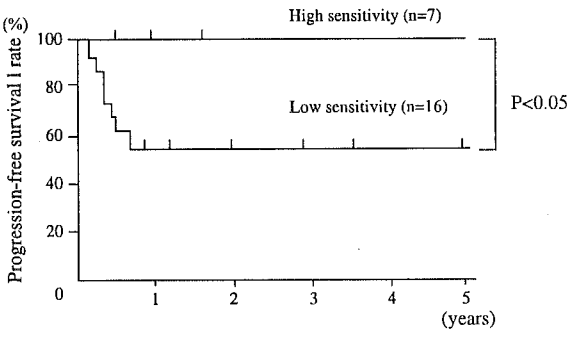

Figure 2. Prognosis of patients in stage III or IV. The 5-year progression-free survival rate of stage III or IV patients with highly sensitive tumors was 100%, while it was only 42.9% when the tumors were of low sensitivity, showing a significant difference between the two groups (Cox–Mantel test, $P < 0.05$).

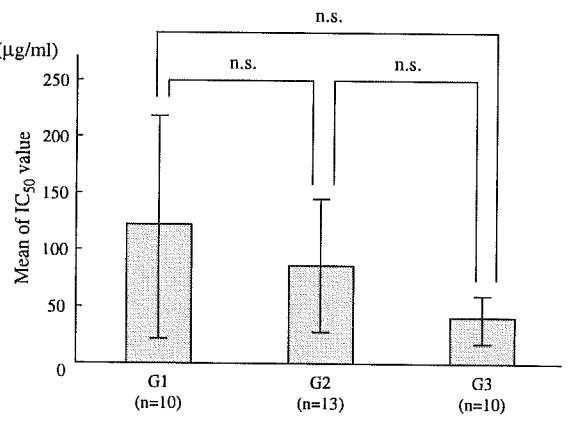

Figure 3. Cisplatin sensitivity of endometrioid adenocarcinoma with different grades of histologic differentiation. There was no significant difference of cisplatin sensitivity in the HDRA between different grades of endometrioid adenocarcinoma (G1, 10 patients; G2, 13 patients; and G3, 10 patients) (Welch's test).

endometrial cancer. The tumor evaluation rate may have been higher with the HDRA than the other methods because the original tissue architecture and tumor cell viability are maintained[18].

The cutoff IC_{50} value for the HDRA should ideally have been set by determining the value giving the maximum accuracy in comparison with the actual clinical response, but there were too few patients with measurable lesions in this series. Instead, a cumulative efficacy curve was plotted from the IC_{50} values obtained, and the cutoff value was based on the previously reported clinical response rate for cisplatin monotherapy (23 μg/mL). With this method, the accuracy rate of the HDRA was 87.5% in the eight patients who had measurable lesions. It fell to 75% when the cutoff IC_{50} value was set between 23 and 33 μg/mL or between 16 and 21 μg/mL. Accordingly, it seems appropriate to set the cutoff IC_{50} value at 23 μg/mL, but it will be necessary to confirm this in a larger number of patients with evaluable lesions. In ovarian cancer patients in whom the clinical response was evaluable, we previously investigated the extent of agreement between tumor sensitivity determined by the HDRA and the actual clinical response, and we confirmed that the cutoff value of the assay was appropriate because it was almost identical to that determined on the basis of the reported clinical response to cisplatin monotherapy[19]. Even with a cutoff IC_{50} value that was based on data reported previously, the accuracy rate of the HDRA was high in our patients with measurable lesions, as has been reported before[18,19].

Because the postoperative recurrence rate is very low in patients with stage I or II endometrial cancer[17], it was expected to be difficult to evaluate the efficacy of adjuvant therapy in such patients. Therefore, we evaluated the efficacy of chemotherapy based on recurrence/progression in patients with stage III or IV disease alone. Among them, we found that chemotherapy could prevent recurrence of advanced cancer, provided that the HDRA showed a high sensitivity. In the low-sensitivity group, recurrence tended to occur earlier, presumably because of the inadequate efficacy of chemotherapy. According to Blackledge[20], the response rate to repeat chemotherapy containing cisplatin was less than 10% among patients with ovarian cancer that recurred during initial cisplatin treatment or in whom the interval between initial treatment and recurrence was only 3–6 months. In the present study, recurrence occurred within 6 months of treatment in 87.5% (seven out of eight) of the patients from the low-sensitivity group with recurrence. These findings suggested tumor resistance to cisplatin, so the results of the HDRA may be related to the clinical response to chemotherapy. Because the prognosis of stage III or IV patients was better in the high-sensitivity group than the low-sensitivity group, it may be possible to improve survival by selection of cisplatin therapy based on the results of HDRA evaluation.

In the present study, there was no significant difference in cisplatin sensitivity between the different histologic grades of endometrioid adenocarcinoma, but the mean IC_{50} value decreased as the tumor grade became lower. This might have been due to more rapid growth of cancer cells in these lesions, reflecting the aggressiveness of low-grade tumors. To clarify this point, investigation of a larger number of tumors will be necessary.

Since the response rate to multiple-agent chemotherapy is usually only around 40–50%[21], this rate might be increased if the indications for chemotherapy could be determined by a drug sensitivity test that accurately predicted the effective agents. Selection of more effective drugs by sensitivity testing may also improve the quality of life by avoiding adverse reactions caused by ineffective agents.

No consensus has been reached as to whether chemotherapy or irradiation should be given postoperatively to patients with endometrial cancer, but the HDRA may help to determine which is more useful in the future, although it would be necessary to perform a randomized comparative clinical study. Since most of the patients in the present study received CAP therapy, the HDRA should possibly have been performed for all drugs in the chemotherapy regimen. Because of interactions among drugs, however, the sum of the sensitivities to the individual agents does not

necessarily reflect the efficacy of multiple-agent chemotherapy. Our finding that the sensitivity to cisplatin shown by the HDRA was correlated with the clinical response suggests that cisplatin is a key drug for endometrial cancer and that this test is useful to predict the prognosis when platinum-based therapy is given. Cisplatin is still widely used in many chemotherapy regimens for endometrial cancer, even though new drugs such as taxanes or topoisomerase inhibitors have been developed, so it remains a key drug in clinical practice. There was no significant difference in the cisplatin sensitivity of endometrioid adenocarcinoma with different levels of differentiation, presumably because of the relatively small number of tumors studied.

In conclusion, there was a strong correlation between sensitivity to cisplatin determined by the HDRA and the clinical response of endometrial cancer. Accordingly, this method may be useful for predicting the efficacy of chemotherapy and may provide useful information when selecting chemotherapy or radiotherapy as postoperative adjuvant therapy.

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Lactation and Risk of Endometrial Cancer in Japan: A Case-Control Study

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OKAMURA, C., TSUBONO, Y., ITO, K., NIIKURA, H., TAKANO, T., NAGASE, S., YOSHINAGA, K., TERADA, Y., MURAKAMI, T., SATO, S., AOKI, D., JOBO, T., OKAMURA, K. and YAEGASHI, N. *Lactation and Risk of Endometrial Cancer in Japan: A Case-Control Study.* Tohoku J. Exp. Med., 2006, **208** (2), 109-115 —— The incidence of endometrial cancer is rapidly increasing in Japan. Although the risk factors in European populations have been well described, there are few epidemiologic studies regarding risk factors for endometrial cancer in Japanese women. This hospital-based case-control study among Japanese women was carried out from 1998 to 2000. The cases were selected from women with endometrial cancer (n =155), and the controls selected from women attending the university gynecological outpatient clinic for cervical cancer screening (n = 96). Subjects were interviewed to ascertain breast feeding practices, contraceptive usage, as well as potential risk factors for endometrial cancer. We observed a lower risk of endometrial cancer associated with oral contraceptive (OC) and a higher risk associated with higher body mass index (BMI), and older ages at first and last delivery. Gravidity reduced odds ratio (OR) for endometerial cancer to 0.34 (95% confidence interval [CI] 0.13-0.92). Compared with parous women who had never breastfed, the multivariate OR for women with a history of breastfeeding was 0.37 (95% CI, 0.17-0.82). Additionally, a greater lapse of time since breastfeeding increased OR for endometrial cancer by over three times. In conclusion, the present study has indicated that breastfeeding reduces the risk of endometrial cancer in Japanese women.
——— endometrial cancer; breastfeeding; risk factor; case-control study

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Age-adjusted incidence rates for endometrial cancer have doubled during the past two decades among Japanese women. The rising incidence possibly may be due to changes in lifestyle, or changes in reproductive factors such as childbearing and contraception, as these characteristics have been associated with endometrial cancer risk in Western populations. In Western countries, there is considerable evidence that reproductive factors play a role in the etiology of endometrial cancer. Nulliparity and obesity have been associated with a higher risk, whereas oral contraceptive (OC) use has been associated with a lower risk (Kirschner et al. 1981; Kelsey et al. 1982; Zumoff 1982; Austin et al. 1991; Schapira et al. 1991; Brinton et al. 1992; Shu et al. 1992; Kalandidi et al. 1996; McPherson et al. 1996; Iemura et al. 2000; Herrinton et al. 2001). A few studies have examined the association between breastfeeding and endometrial cancer risk (Rosenblatt and Thomas 1995; Salazar-Martinez et al. 1999; Newcomb and Trentham-Dietz 2000); however, the findings from these studies are inconsistent.

The reproductive characteristics of Japanese women, however, are different from those of Western populations. For instance, 15%, 36%, and 59% of contraceptive-using women choose OCs in the United States, France, and Germany, respectively, whereas the prevalence of OC use is only 1.5% among Japanese women who use contraception. Only 1.8% of Japanese women older than 50 years have used hormone replacement therapy (HRT), whereas the prevalence of HRT usage is 53% among US women aged 50-59 years. These differences make it difficult to generalize findings obtained in Western studies to Japanese women. There have, however, been a few studies evaluating risk factors for endometrial cancer in Japanese women (Inoue et al. 1994; Hirose et al. 1996, 1999). Therefore, this study was undertaken to further characterize endometrial cancer risk factors in Japanese population.

Subjects and Methods

This case-control study was a collaborative investigation in three areas of Japan (Tokyo, Kanagawa, and Miyagi). Cases were accrued from three university hospitals from January 1, 1998, through December 31, 2000. Eligible cases included Japanese women between 20 and 80 years of age who underwent surgery for a diagnosis of endometrioid endometrial cancer confirmed by histology. The cases resided in defined geographic catchment areas, and had not received treatment previously. One hundred sixty seven cases were eligible for the study and 12 subjects refused to participate. Thus, 155 (93%) of the eligible cases participated. Stage distribution of the cases was as follows: stage I, n = 104; stage II, n = 14; stage III, n = 33; and stage IV, n = 4.

The controls were selected from women who attended gynecologic outpatient clinics in the university hospitals for cervical cancer screening. Controls included only women with intact uteri. Ninety six women were included as controls; however, 9 women refused participation (participation rate, 91%). Cases and controls were not matched in terms of age or other variables.

The protocol for this study was approved by the Ethics Committee at Tohoku University Graduate School of Medicine (Sendai, Japan).

Gynecologists interviewed the cases and controls using a standard questionnaire asking about demographic information, medical history, cigarette use, and reproductive history (parity, gravidity, and ages at first pregnancy, last delivery, menarche, menopause, and lactation). Body mass index (BMI) was calculated based on self-reports of weight (kg)/height $(m)^2$. The distribution of continuous variables was examined among cases and controls and divided into two or three categories.

To estimate the risk of endometrial cancer associated with various factors, we calculated age-adjusted and multivariate odds ratio (ORs) along with 95% confidence interval (CI) using unconditional logistic regression analysis. Statistical Analysis System (SAS Institute, Cary, NC, USA) software was used for all statistical analyses.

Results

The mean ages of cases and controls were 56.1 years and 49.6 years, respectively. Table 1 presents age-adjusted ORs and 95% CIs of the selected variables for the risk of endometrial cancer. Higher BMI was associated with higher risk (p = 0.01). OC use was associated with a lower risk of disease (OR, 0.16; 95% CI, 0.04-0.66), although only three cases and ten controls used OCs. Intra-uterine device use, history of HRT, smoking, sterility, hypertension, diabetes mellitus,

TABLE 1. *Baseline characteristics of cases and controls*

Caracteristics	Cases	%	Controls	%	Age-adjusted OR	95% CI	*p* value
Age (years)							
< 45	15	9.7	39	40.6			
45-55	52	33.6	23	24			
55-65	55	35.4	24	25			
≧ 65	33	21.3	10	10.4			
BMI (kg/m^2)							
< 20.04	36	23.3	26	27.1	1.00		
20.04-21.63	27	17.4	35	36.5	0.47	0.22-0.99	
21-64-23.92	45	29.0	20	20.8	1.24	0.58-2.67	
≧ 23.93	47	30.3	15	15.6	1.92	0.86-4.30	0.01
Oral contraceptive use							
Never	152	98.1	86	89.6	1.00		
Ever	3	1.9	10	10.4	0.16	0.04-0.66	0.01
IUD use							
Never	148	95.5	90	93.8	1.00		
Ever	7	4.5	6	6.2	0.54	0.17-1.71	0.29
HRT use							
Never	132	85.16	85	88.5	1.00		
Ever	23	14.84	11	11.5	1.4	0.63-3.14	0.41
Cigarette smoking							
Never	126	81.3	77	80.2	1.00		
Ever	29	18.7	19	19.8	1.30	0.65-2.61	0.52
Sterility							
Never	143	92.3	87	90.6	1.00		
Ever	12	7.7	9	9.4	0.81	0.31-2.11	0.66
Hypertension							
Never	115	74.2	87	90.6	1.00		
Ever	40	25.8	9	9.4	2.15	0.95-4.86	0.45
Diabetes mellitus							
Never	139	89.7	92	95.8	1.00		
Ever	16	10.3	4	4.2	1.82	0.56-5.92	0.32
Personal cancer history							
Never	139	89.7	92	96.8	1.00		
Ever	16	10.3	4	4.2	1.78	0.55-5.73	0.33

and personal cancer history were not associated with risk. There were 20 persons who had personal cancer history. Among them 11 persons had breast cancer and the remaining nine persons had cancer history at various sites, such as colon cancer, rectal cancer, thyroid cancer, gastric cancer, lung cancer, and ovarian cancer. Four of the 20 persons had hormone therapy.

Table 2 shows the ORs for the association of endometrial cancer with reproductive factors.

TABLE 2. *Multivariate Odds Ratio and 95%Confidence Intervals for Endometrial Cancers-According to Reproductive Factors*

Variables	Cases	%	Controls	%	OR*	95% CI	*p* value
Menopausal status							
Pre	51	32.9	55	57.3	1.00		
Post	104	67.1	41	42.7	0.91	0.39-2.14	0.82
Gravidity							
Never	20	12.9	9	9.4	1.00		
Ever	135	87.1	87	90.6	0.34	0.13-0.92	0.03
No. of pregnancies							
0	20	12.9	9	9.4	1.00		
1	27	17.4	16	16.7	0.43	0.14-1.33	
2	42	27.1	32	33.3	0.34	0.12-0.97	
≧ 3	66	42.6	39	40.6	0.29	0.10-0.85	0.04
Parity							
Never	36	23.2	21	21.9	1.00		
Ever	119	76.8	75	78.1	0.46	0.22-0.96	0.04
No. of deliveries							
0	36	23.2	21	21.9	1.00		
1	29	18.7	18	18.8	0.45	0.18-1.12	
2	68	43.9	44	45.8	0.47	0.21-1.04	
≧ 3	22	14.2	13	13.5	0.44	0.16-1.20	0.1
Age at first delivery**							
≦ 24	43	36	11	14.7	1.00		
25-26	36	30.3	23	30.7	0.45	0.18-1.10	
27-29	21	17.7	23	30.7	0.30	0.12-0.78	
≧ 30	19	16	18	24	0.35	0.13-0.96	0.05
Age at last delivery**							
≦ 25	23	19.3	6	8	1.00		
26-30	40	33.6	25	33.3	0.48	0.16-1.45	
31-33	39	32.8	26	34.7	0.45	0.15-1.36	
≧ 34	17	14.3	18	24	0.28	0.08-0.94	0.02

* OR adjusted for age, BMI, and oral contraceptive use.
** Parous women only.

The ORs were adjusted for age, BMI, and OC use. Gravidity was inversely associated with endometrial cancer risk. Women who reported ever being pregnant had only one third the risk of endometrial cancer compared with women who had never been pregnant (OR, 0.34; 95% CI, 0.13-0.92, $p = 0.03$). Women who reported three or more pregnancies had about one third the risk of women with no pregnancies (OR, 0.29; 95% CI, 0.10-0.85).

Parity was also inversely associated with endometrial cancer risk. Women who reported ever having delivery had about one half the risk of endometrial cancer compared with women who had never delivered (OR, 0.46; 95% CI, 0.22-0.96, $p = 0.04$). Higher age at the first or last deliveries was associated with a lower risk for endometrial cancer ($p = 0.05$, $p = 0.02$). Age at menarche, menopausal status, age at menopause, history of dysmenorrhea, and history of abortion were not associated with risk (data not shown).

Only parous women, representing 119 cases and 75 controls, were included in the analysis of the association between breastfeeding and endometrial cancer risk presented in Table 3. Table 3 also showed the age distribution of both cases and control and that of the lapse of the last breast feeding. The ORs were adjusted for age, BMI, and OC use as shown in Table 3. Compared with parous women who had never breastfed, the multivariate odds ratio for women who had ever breastfed was 0.37 (95% CI: 0.17-0.82, $p = 0.013$). A greater lapse of time since breastfeeding concluded was directly associated with an increased risk of endometrial cancer (OR of 20-29 years, 3.10, 95% CI: 1.14-8.48, and OR of 30 or longer, 3.85, 95% CI: 1.00-14.84, $p = 0.045$). Then, we analyzed the association between frequency or duration of breastfeeding and endometrial cancer risk, but did not find any significant association (data not shown).

Discussion

In this hospital-based case-control study among Japanese women, we observed a lower risk of endometrial cancer associated with OC use and gravidity, and a higher risk associated with higher BMI, older ages at first and last delivery and number of pregnancies. These findings were consistent with data obtained in prior Japanese studies (Inoue et al. 1994; Hirose et al. 1996, 1999). In contrast to the study by Inoue et al. (1994), our study failed to demonstrate an association between a history of hypertension, diabetes mellitus, or cancer.

Our study also demonstrated a reduction in the risk of endometrial cancer associated with breastfeeding. The proportion of never breastfeeding (35.3%) in endometrial cancer cases was larger than that in control, but the risk was signifi-

Table 3. *Multivariate Odds Ratio for Endometrial Cancers in relation to breastfeeding and age among parous women*

Variables	Cases (*n*)					Controls (*n*)					OR*	95% CI	*p* value
	Total	<45	45-55	55-65	65≦	Total	<45	45-55	55-65	65≦			
breastfeeding													
Never	42	1	10	24	7	11	3	3	2	3	1.00		
Ever	77	1	31	26	19	64	25	14	18	7	0.37	0.17-0.82	0.013
Years since last breastfed**													
1-19	12	1	9	1	1	33	24	7	2	0	1.00		
20-29	31	0	17	12	2	20	1	7	11	1	3.10	1.14-8.48	
≧30	34	0	5	13	16	11	0	0	5	6	3.85	1.00-14.84	0.045

* Adjusted for age, BMI and oral contraceptive use.
** Ever breastfed women only.