Favourable Prognosis with Modified Dosing of Docetaxel and Cisplatin in Japanese Patients with Ovarian Cancer

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Abstract. *Background: The long-term efficacy and safety of docetaxel/cisplatin as first-line chemotherapy in Japanese patients was evaluated in order to find an optional regimen for ovarian cancer. Patients and Methods: Women with surgically resected stage Ic-IV epithelial ovarian cancer were treated with docetaxel 70 mg/m^2 and cisplatin 60 mg/m^2 every 4 weeks. Results: Ninety women were enrolled of whom 89 (median age, 54 years) received a median of 6 cycles (range 1 to 9). With a median 38 months' follow-up, median progression-free survival was 28 months (95% lower confidence interval, 24 months) in 60 patients with stage III-IV disease. The overall response rate for 20 patients was 45%. Neutropenia was the most common (67%) grade 3/4 toxicity. Major grade 3/4 nonhaematological toxicities were gastrointestinal toxicities (≤11%) and fatigue (8%). No grade 3/4 neurotoxicity was observed. Conclusion: The combination of docetaxel/cisplatin is a regimen with favourable progression-free survival for ovarian cancer.*

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Combination therapy with paclitaxel and a platinum-agent has become first-line treatment for advanced ovarian cancer following the results of phase III studies (1-5). However, side-effects associated with the paclitaxel/carboplatin regimen, such as thrombocytopenia and neurotoxicity, remain a concern (3-5).

A new cisplatin-based regimen is essential as an optional therapy for ovarian cancer. Previous phase III studies comparing paclitaxel/cisplatin and paclitaxel/carboplatin showed non-inferiority for the carboplatin regimen. Therefore paclitaxel/cisplatin has not been used in recent studies due to hydration requirements with cisplatin and gastrointestinal toxicity (3-5). Moreover, the paclitaxel/cisplatin combination was more inconvenient due to the 24-hour continuous infusion required for administration. Considering that taxane/platinum combinations have become the standard regimen for advanced ovarian cancer, evaluation of alternative taxanes such as docetaxel combined with cisplatin is needed in order to broaden treatment options.

Previously, two phase II studies of docetaxel/cisplatin for ovarian cancer were conducted in the UK and France, showing that progression-free survival (PFS) and overall survival (OS) were modest (6, 7). However, accurate effects of docetaxel/cisplatin on survival are still unclear as PFS or OS were not evaluated as primary endpoints. Moreover, 33% of patients were not able to complete the planned 6 cycles of docetaxel (75-85 mg/m^2) and cisplatin (75 mg/m^2) in the UK

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study. The maximum approved dose of docetaxel is 70 mg/m^2 in Japan (8). A small randomized feasibility study in Japanese patients suggested that, in contrast to the overseas experience, cisplatin at a dose of 60 mg/m^2 has similar effects to carboplatin AUC 5 when combined with docetaxel (9). Therefore, to evaluate the effects on survival in patients with ovarian cancer, an alternative regimen of docetaxel/cisplatin was evaluated in this multicentre phase II study using fixed doses of docetaxel (70 mg/m^2) and cisplatin (60 mg/m^2) in patients with International Federation of Gynaecology and Obstetrics (FIGO) stage Ic-IV epithelial ovarian cancer.

Patients and Methods

Ethical considerations. This multicentre, open-label, single-arm, phase II clinical study was conducted at 11 institutions throughout Japan in accordance with the Declaration of Helsinki 2000. The protocol was reviewed and approved by the institutional review board of each participating institution and written informed consent was obtained from all patients prior to the study.

Eligibility. Women aged 20-74 years who had histologically confirmed epithelial ovarian cancer with FIGO stage Ic-IV were eligible. Other inclusion criteria were: Eastern Cooperative Oncology Group performance status of 0-2; an anticipated life expectancy of at least 3 months; white blood cells 4,000-12,000/mm^3; neutrophils ≥ 2,000/mm^3; platelets ≥100,000/mm^3; haemoglobin ≥9.0 g/dl; total bilirubin ≤1.5 mg/dl; aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ≤1.5 × the upper normal limit (UNL); alkaline phosphatase ≤2.5 × UNL; creatinine ≤1.5 mg/dl; and creatinine clearance ≥60 ml/min.

Patients who received exploratory laparotomy without debulking were excluded. Prior systemic therapy (chemotherapy or biological response modifiers) or radiation therapy was not allowed. Patients were also excluded if they had confirmed infection; fever (≥38°C); serious concomitant illness such as severe cardiovascular disease, uncontrolled diabetes, malignant hypertension and haemorrhagic disease; active concomitant malignancy; brain metastasis; interstitial pneumonia or lung fibrosis confirmed by chest X-ray or computed tomography; pleural or peritoneal effusion that required treatment; pericardial effusion; motor paralysis, peripheral neuropathy or oedema of grade 2 or more; history of severe drug allergy; or had previously received long-term corticosteroid therapy. Pregnant or lactating women were also excluded.

Treatment plan. Intravenous docetaxel (70 mg/m^2 over 1-2 hours) and cisplatin (60 mg/m^2 over 2-3 hours) were administered on day 1 every 4 weeks. Patients were given adequate hydration before and after treatment. Patients received at least 6 cycles of treatment unless disease progression or unacceptable toxicity was observed. If any of the following toxicities was observed, treatment was withheld until recovery was confirmed: neutrophils <2,000/mm^3; platelets <100,000/mm^3; serum creatinine >1.5 mg/dl; creatinine clearance <60 ml/min; AST or ALT > 2.5 UNL; body temperature ≥38°C; performance status ≥3; neuropathy of grade 2 or more; oedema of grade 2 or more. If any of the following was observed, the dose of docetaxel was reduced to 60 mg/m^2 in subsequent cycles: grade 4 neutropenia lasting at least 5 days; grade 4 neutropenia with fever (≥38°C) lasting at least 3 days. Prophylactic anti-emetic therapy was allowed. If oedema was observed in the previous cycle, treatment with corticosteroid was given, but corticosteroids were not routinely provided as premedication for docetaxel. Granulocyte colony-stimulating factor was administered for subsequent cycles if grade 4 neutropenia or febrile neutropenia was observed.

Study end-points. The primary endpoint was to evaluate PFS among patients with FIGO stage III-IV ovarian cancer. PFS was defined as the time from the start of study treatment until objective tumour progression or death for any reason. The secondary end-points were tumour response classified according to the Response Evaluation Criteria in Solid Tumours (RECIST) (10), OS, feasibility (the percentage of patients who completed at least 6 cycles of combination treatment) and adverse events. The adverse events were assessed according to National Cancer Institute Common Toxicity Criteria (version 2).

Statistical analysis. The sample size calculation was based on an estimated median PFS of 24 months in patients with stage III-IV disease. To demonstrate median PFS of at least 16 months in this patient population, it was planned to enroll 60 patients with stage III-IV (type I error level = 0.05, one sided; type II error level =0.2). In addition, in order to fully evaluate the safety of docetaxel/cisplatin in patients with ovarian cancer, enrolment of up to 30 additional patients with stage Ic-II disease was allowed. OS and PFS were analysed using the Kaplan-Meier method. All analyses were performed with JMP® version 5.1.2 (SAS Institute Inc., Cary, NC, USA).

Results

Patients. Between November 2001 and November 2004, 90 patients were enrolled. One patient was excluded from all analyses because she was treated with a chemotherapy regimen not specified in the protocol. The median age was 54 years (range, 34 to 72) and the majority of patients had performance status of 0 or 1. Twenty-nine patients (33%) were stage Ic-II and 60 patients (67%) were stage III-IV (Table I). Fifteen patients (17%) had distant metastasis defined as stage IV disease.

Treatment and toxicities. Patients received a median of 6 cycles (range, 1 to 9) and 66 patients (74%) received more than 6 cycles. A total of 14 patients (16%) discontinued treatment due to adverse events. Reasons for discontinuation were deterioration in renal function, 4 patients; neutropenia, 3; lymphocoele, 2; diarrhoea, oedema, ventricular premature contraction, colorectal cancer, and unidentified toxicity in 1 patient each. Leucopoenia and neutropenia were the most common haematological toxicities. Grade 3/4 haematological events included neutropenia (60 patients, 67%), febrile neutropenia (15, 17%), and leucopoenia (9, 10%). Grade 3/4 thrombocytopenia and anaemia were not observed (Table II).

The most common nonhaematological toxicities were anorexia, fatigue, vomiting, nausea and alopecia. Grade 3/4

Table I. *Baseline patient characteristics.*

Characteristic	Stage* Ic-II (n=29)	Stage* III-IV (n=60)	Total (n=89)
Median (range) age, years	51 (34 to 70)	54 (37 to 72)	54 (34 to 72)
ECOG Performance status, n (%)			
0-1	28 (97)	60 (100)	88 (99)
2	1 (3)	0 (0)	1 (1)
Cell type, n (%)			
Serous	8 (28)	34 (57)	42 (47)
Clear cell	12 (41)	8 (13)	20 (22)
Other	9 (31)	18 (30)	27 (30)
Postoperative residual tumour (>1 cm)	0 (0)	35 (58)	35 (39)

*International Federation of Gynaecology and Obstetrics (FIGO) classification. ECOG, Eastern Cooperative Oncology Group.

Table II. *Treatment delivery and adverse events (n=89).*

Treatment delivery	Number (%) of patients	
Number of treatment cycles		
<6 cycles	23 (26)	
≥6 cycles	66 (74)	
Reason for terminating treatment (<6 cycles)		
Adverse events	14 (16)	
Disease progression	5 (6)	
Others	4 (4)	
Dose reduction	5 (6)	
Adverse events	**Number (%) of patients**	
	All grades*	Grade 3 or 4*
Haematological		
Leucopenia	74 (83)	9 (10)
Neutropenia	64 (72)	60 (67)
Anaemia	31 (35)	0 (0)
Thrombocytopenia	37 (42)	0 (0)
Neutropenic complications		
Febrile neutropenia	15 (17)	15 (17)
Neutropenia with infection	8 (9)	8 (9)
Nonhaematological		
Anorexia	75 (84)	10 (11)
Fatigue	65 (73)	7 (8)
Vomiting	43 (48)	6 (7)
Nausea	74 (83)	4 (5)
Infection (without neutropenia)	10 (11)	2 (2)
Fever	5 (6)	0 (0)
Diarrhoea	31 (35)	3 (3)
Oedema	19 (21)	2 (2)
Stomatitis	13 (15)	3 (3)
Alopecia	55 (62)	-
Neuropathy	37 (42)†	0 (0)
Ventricular premature contraction	1 (1)	1 (1)
Dyspnoea	1 (1)	1 (1)
AST elevation	34 (38)	2 (2)
ALT elevation	34 (38)	3 (3)
Creatinine elevation	32 (36)	2 (2)

* National Cancer Institute common toxicity criteria version 2. †Grade 2, 4 patients; grade 1, 33 patients. AST, aspartate aminotransferase; ALT, alanine aminotransferase.

events included anorexia in 10 patients (11%), fatigue (7, 8%), vomiting (6, 7%) and nausea (4, 4%). Grade 1 neuropathy was observed in 33 patients (37%) and grade 2 event was observed in 4 (4%), but no grade 3/4 neurotoxicity was observed. Two patients had severe elevation of serum creatinine. There were no treatment-related deaths.

Clinical efficacy. Figure 1 shows PFS and OS in patients with stage Ic-II and III-IV disease after median follow-up of 38 months (range, 4 to 58). The median PFS in patients with stage III-IV disease was 28 months (95% lower confidence interval [CI], 24 months), but median PFS in stage Ic-II patients was not reached due to insufficient events.

Twenty-two patients with stage III and 13 patients with stage IV had suboptimally debulked tumour (≥1 cm residual tumour). Among these, 20 patients had residual measurable lesions as defined by RECIST and were evaluated for clinical response. No patients with stage I-II had measurable disease after surgery. The overall response rate (ORR) was 45% (95% CI, 27-73%): 5 patients (25%) had complete response and 4 (20%) had partial response.

Discussion

For patients with stage III-IV ovarian cancer, the regimen of docetaxel 70 mg/m^2 and cisplatin 60 mg/m^2 every 4 weeks resulted in a median PFS of 28 months, exceeding the expected 24 months. PFS of platinum-taxane doublets in previous randomized studies was 14 and 18 months for suboptimal stage III-IV disease (1, 11) and 19 and 21 months for optimal stage III disease (5). Previous studies of docetaxel/cisplatin (6, 7) demonstrated 12-14 months for PFS. However, PFS was not the primary endpoint of these studies. The UK study described that although median PFS of cohort 2 (starting dose 85 mg/m^2 of docetaxel) was slightly shorter than cohort 1 (75 mg/m^2 of docetaxel), significant differences between cohorts were not proven because of the small patient numbers (6). The results of another study showed a wide 95% CI of 8-20 months for PFS due to the small sample size in the evaluation of the pathological response (7). Although the completion rate of 74% in the presented study was higher than other reports using different doses of docetaxel/cisplatin (66%) (6), it is still slightly lower than paclitaxel/platinum (79-89%) (1, 3-5, 11-13). It may have been possible to increase the

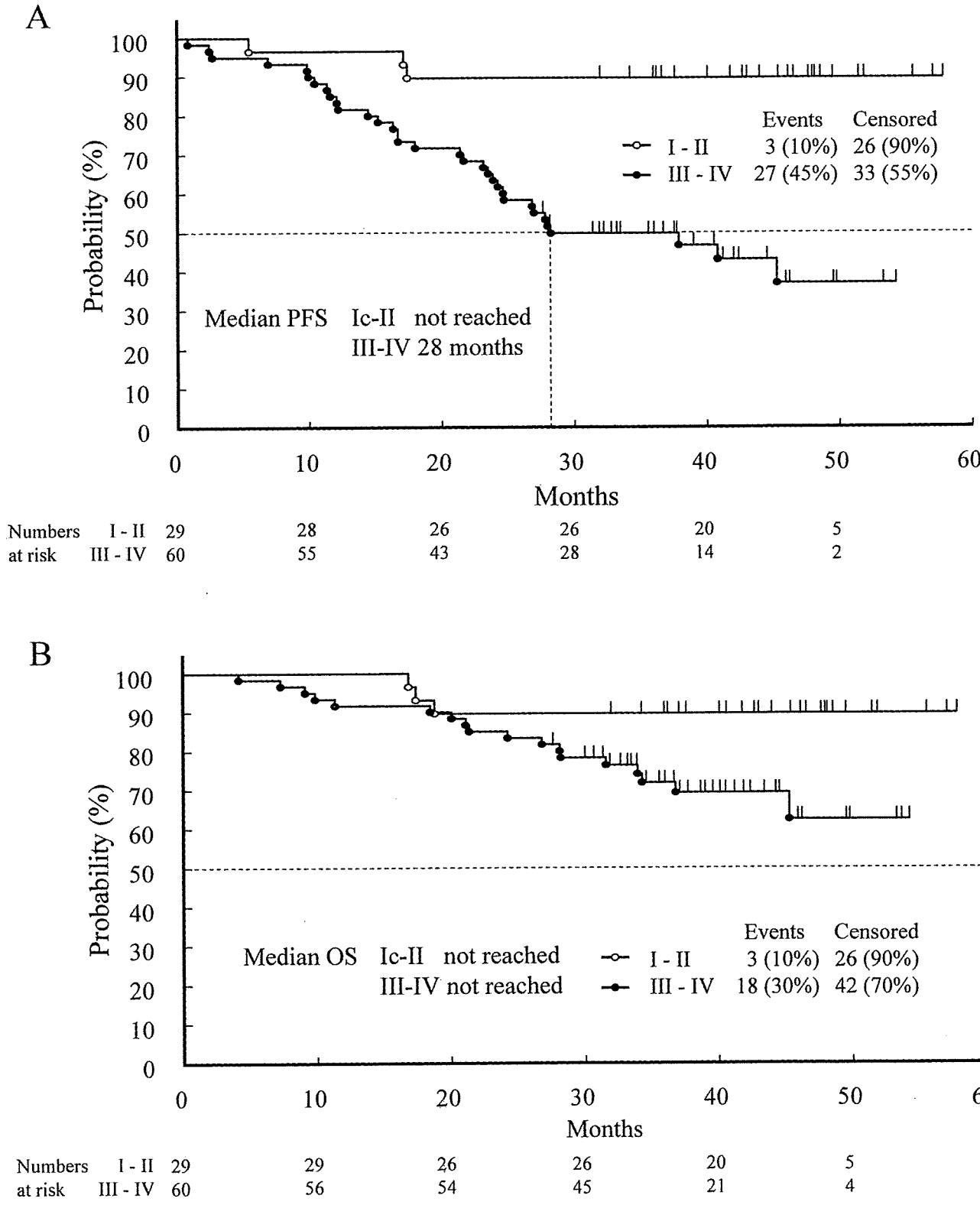

Figure 1. *The cumulative probability of progression-free survival (A) and overall survival (B) estimated by the Kaplan-Meier method for patients with stage Ic-II (○) or stage III-IV (●) disease.*

completion rate for this regimen had the physicians been better acquainted with the toxicity profile of docetaxel/cisplatin, as some cases of treatment termination resulted from physicians' decisions. Since the presented results did not show grade 3/4 thrombocytopenia, neurotoxicity or hypersensitivity reactions and appearance of grade 3/4 gastrointestinal toxicity was similar to paclitaxel/carboplatin, the number of patients who discontinued treatment because of deterioration in renal function was only 4 (4.5%). It is noted that the incidence of febrile neutropenia in this study seems to be higher than that with paclitaxel/carboplatin, however all cases were manageable. Because grade 1 neurotoxicity often appeared, careful observation might be needed for the long-term use of docetaxel/cisplatin.

Thirty-five patients (58%) with suboptimally debulked stage III-IV disease were enrolled in this study. However, only 20 patients were eligible for clinical response evaluation because some residual tumours were smaller than the measurable lesions defined by RECIST. The number of patients with stage IV disease was also small. It must be noted that not only the regimen used in this study but also these differences of the patient characteristics might be a reason for favourable PFS in patients with advanced ovarian cancer. Although care must be taken when comparing the results of this phase II study to those obtained from previous randomized controlled studies, the combination of cisplatin 60 mg/m^2 and docetaxel 70 mg/m^2 showed promising results and could be considered as a safe alternative to paclitaxel/carboplatin as a first-line treatment option. A phase III study in the UK described above evaluated docetaxel as an alternative taxane for combination with carboplatin to develop a more effective regimen than paclitaxel/carboplatin, however docetaxel/carboplatin did not demonstrate superior results (12). Moreover, triplet regimens which added either gemcitabine or liposomal doxorubicin either sequentially or concurrently with combined paclitaxel/carboplatin were compared with this first-line treatment and these new regimens did not show superior survival (14). Development of a new regimen with improvement in survival remains a difficult issue.

According to evidence of chemotherapy for advanced epithelial ovarian cancer, cyclophosphamide/cisplatin should be given when patients are anxious about neurotoxicity and reject paclitaxel/carboplatin therapy (13). However, the present phase II study has shown that docetaxel/cisplatin therapy can be expected to prolong PFS without severe neurotoxicity. It is concluded that the combination of docetaxel/cisplatin is a feasible first-line regimen with favourable efficacy avoiding options of non-taxane/platinum for Japanese patients with epithelial ovarian cancer.

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Combination chemotherapy of oxaliplatin and 5-fluorouracil may be an effective regimen for mucinous adenocarcinoma of the ovary: A potential treatment strategy

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Resistance of ovarian mucinous adenocarcinoma to standard chemotherapy with paclitaxel and carboplatin is associated with poor prognosis, and an effective treatment is needed. The present study aimed to identify an effective chemotherapy for ovarian mucinous adenocarcinoma. Five human ovarian mucinous adenocarcinoma cell lines (MN-1, OMC-1, RMUG-L, RMUG-S, TU-OM-1) were used in this study. Sensitivity of the cells to the anticancer agents was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay, and we assessed drug sensitivity by calculating the assay area under the curve for each agent. Protein expression was confirmed by Western blot analysis. We also examined the efficacy of combination chemotherapy on survival in a xenograft model of nude mice. The IC_{50} to anticancer agents ranged widely. The assay area under the curve indicated that two of five cell lines (MN-1, TU-OM-1) were sensitive to oxaliplatin, 5-fluorouracil and etoposide, and only one (TU-OM-1) was sensitive to 7-ethyl-10-hydroxycamptothecin, which is an active metabolite of camptothecin. All cell lines were resistant to cisplatin and paclitaxel. The combination of oxaliplatin and 5-fluorouracil resulted in additive or synergistic effects on all cell lines. The combination of oxaliplatin and 5-fluorouracil significantly prolonged survival in a ovarian mucinous adenocarcinoma xenograft model of nude mice. Protein expression levels of the excision repair cross-complementation group 1 were lower in oxaliplatin sensitive cell lines. Exposure to 5-fluorouracil down-regulated cross-complementation group 1 expression in ovarian mucinous adenocarcinoma cells. We conclude that combination chemotherapy consisting of oxaliplatin and 5-fluorouracil was an effective treatment for ovarian mucinous adenocarcinoma and may be a pivotal candidate for a novel treatment strategy. (*Cancer Sci* 2009; 100: 546–551)

Mucinous adenocarcinoma of the ovary (MAC) is the third most common type of epithelial ovarian cancers (EOC), comprising 10 to 12% of EOC. MAC appears to have a distinctly different clinical behavior from that of other EOC.[1–3] Several studies show that MAC is often diagnosed at an early stage, and therefore, confers a relatively good prognosis.[1–3] However, advanced MAC has a poorer prognosis than other histopathologic subgroups.[4–6] MAC's low response (26–42%) to conventional platinum-based chemotherapy is associated with poor prognosis because chemosensitivity is one of the main prognostic factors for patients with advanced EOC.[4–9] Although MAC is known to be resistant to platinum- and taxane-based chemotherapy, patients with EOC are usually treated with this first-line chemotherapy regimen. A novel treatment strategy for advanced MAC is urgently needed.

Recently, new agents for EOC have been developed, including oxaliplatin (L-OHP), etoposide (VP-16) and camptothecin (CPT-11).[10] L-OHP (1R,2R-diaminocyclohexane oxalatoplatinum [II]) is a 1,2-diaminocyclohexane (DACH) platinum compound with a partial lack of cross-resistance to platinum analogs, cisplatin (CDDP) and carboplatin.[11–13] VP-16, which inhibits the activity of DNA topoisomerase II, is used as a second-line treatment for patients with platinum-resistant EOC.[14] CPT-11, which inhibits topoisomerase I by forming stable topoisomerase I–DNA cleavable complexes[15] has been studied in relapsed EOC.[16] The thymidylate synthase inhibitor, 5-fluorouracil (5FU), was reported to be an active agent for recurrent EOC.[17,18]

We conducted the present study to identify an effective chemotherapy for MAC.

Materials and Methods

Cell lines and cell cultures. The five MAC cell lines used in the present study (MN-1, OMC-1, RMUG-L, RMUG-S, TU-OM-1) were obtained as follows: MN-1 from Dr Yasuhiko Kiyozuka (Kansai Medical University, Osaka, Japan), OMC-1 from Dr Tsuyoshi Saito (School of Medicine, Sapporo Medical University, Sapporo, Japan), and RMUG-L and RMUG-S from Dr Daisuke Aoki (Keio University, Tokyo, Japan). TU-OM-1 was established by our department. These cell lines were maintained in D-MEM/Ham's F-12 medium (Wako, Osaka, Japan) with 10% fetal bovine serum, 100 IU/mL penicillin and 50 μm/mL streptomycin in a humidified atmosphere containing 5% CO_2 at 37°C.

Dose–response studies. The sensitivity of the cell lines to the anticancer agents was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-dyphenyltetrazolium bromide (MTT) assay.[19] Briefly, cells were diluted with culture medium to the seeding density of $1–3 \times 10^4$ cells/mL, plated on 96-well tissue culture plates at 100 μL/well (Sumitomo Bakelite, Tokyo, Japan), and incubated at 37°C overnight. The next day, the cells were treated continuously with 20 μL of various concentrations of the anticancer agents to obtain a dose–response curve for each agent. Each drug concentration was as follows: 1–100 μM CDDP (Sigma, St. Louis, MO, USA), 0.1–50 μM 5FU (Wako), 1–100 μM L-OHP (Wako), 0.01–10 μM paclitaxel (PTX) (Sigma), 0.01–10 μM 7-ethyl-10-hydroxycamptothecin (SN38) (Yakult Honsha Co., Tokyo, Japan), which is an active metabolite of CPT-11, and 0.1–100 μM VP-16 (Sigma).

After incubation for 72 h, 10 μL MTT solution (5 mg/mL) (Sigma) was added to each well, and the plates were incubated for another 4 h. At the end of that incubation, 100 μL

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dimethylsulfoxide (Sigma) was added to each well to solubilize the MTT formazan product. Absorbance at 570 nm was measured with a microplate reader (model 550; Bio-Rad, Richmond, CA, USA). Growth inhibition was calculated as the percentage of viable cells compared with untreated cultures.

L-OHP or CDDP was combined with 5FU, PTX, SN38 and VP-16 at a fixed ratio that spanned the individual (IC_{50}) of each drug. The 50% inhibitory concentration of a substance (IC_{50}) was determined on the basis of the dose–effect curves, using a standard MTT assay. Median-effect plot analyses and calculation of the combination index (CI) were analyzed by the method of Chou and Talalay.[20] CalcuSyn software (Biosoft, Cambridge, UK) was used to analyze data from the MTT assays in which cells were exposed to agents alone or in combination with anticancer drugs. The computer program provides a measure of the combined agents in an additive or synergistic manner. Chou and Talalay[20] defined CI, which assesses synergism (CI < 0.9), additive behavior (CI = 0.9–1.1) or antagonism (CI > 1.1).

To assess drug sensitivity, the assay area under the curve (AUC) was calculated by means of the following formula: initial concentration $\times t^{1/2} \times 1.44\{1 - \exp(-0.693 \times 72/t^{1/2})\}$. Initial concentration was determined based on the IC_{50} of each agent in the present study and $t^{1/2}$ is the *in vitro* half-life of the drug at 37°C.[21] The half-lives of CDDP and VP-16 are 18.5 and 60 h, respectively.[22] We confirmed that the half-life of L-OHP was 23 h. Those of 5FU, PTX and SN38 were reported as stable in serum-containing medium at 37°C in the presence of 5% CO_2; therefore, the assay AUC of those drugs were calculated as the drug concentration × 72 h.[23] The assay AUC was then compared with the clinically achievable AUC with a standard dose of each drug. If the calculated assay AUC was less than the clinically achievable AUC, the drug was defined as sensitive.

Western blot analysis. Cells were washed twice with phosphate-buffered saline (PBS) and then lyzed in lysis buffer (50 mM Tris-HCl, 125 mM NaCl, 0.1% Nonidet P-40, 5 mM ethylenediaminetetraacetic acid, 50 mM NaF, 0.1% phenylmethylsulfonyl fluoride, protease inhibitors (Protease Inhibitor Cocktail Set I; Calbiochem, Darmstadt, Germany). Protein concentrations were measured against a standardized control by using a protein assay kit (Bio-Rad Laboratories, Hercules, CA, USA). A total of 50 μg protein was separated by electrophoresis on a 10% polyacrylamide gel and transferred to a polyvinylidene difluoride membrane (Millipore, Bedford, MA, USA). The specific antibodies used were rabbit anti-Akt antibody (1:1000 dilution; Cell Signaling Technology, Beverly, MA, USA), rabbit antiphospho-Akt (serine 473) antibody (1:1000 dilution; Cell Signaling Technology), rabbit anti-MEK1 antibody (1:1000 dilution; Cell Signaling Technology), rabbit antiphospho-MEK1 (threonine 286) antibody (1:500 dilution; Cell Signaling Technology), rabbit anti-Bcl2 antibody (1:1000 dilution; Cell Signaling Technology), rabbit anti-Bcl-xL (1:1000 dilution; Cell Signaling Technology), mouse anti-ERCC1 antibody (1:100 dilution; Abcam), mouse anti-XPF antibody (1:100 dilution; Abcam), mouse anti-XRCC1 antibody (1:500 dilution; Abcam) and mouse anti-Actin antibody (1:1000 dilution; Sigma). These were visualized with secondary antirabbit or antimouse IgG antibody coupled with horseradish peroxidase, using enhanced chemiluminescence according to the manufacturer's recommendation.

Annexin V staining. The Annexin V–PE Reagent (BioVision Research Products, Mountain View, CA, USA) was used to assess apoptosis in terms of the externalization of phosphatidylserine residues, according to the specifications of the manufacturer. Briefly, cells were washed twice with cold PBS and once with 1 × Annexin V binding buffer (10 mmol/L HEPES/NaOH [pH 7.4], 140 mmol/L NaCl, 2.5 mmol/L $CaCl_2$). The cells were then stained with Annexin V–PE diluted 1:10 in 1 × Annexin V binding buffer for 15 min at room temperature. Finally, the cells were washed with 1 × Annexin V binding buffer, then 500 μL 1 × Annexin V binding buffer was added to each well and the cells analyzed with a flow cytometer (Olympus, Tokyo, Japan).

Table 1. IC_{50} values to anticancer agents

Cell lines	IC_{50} (μM)					
	CDDP	5FU	L-OHP	PTX	SN38	VP-16
MN-1	12.22	0.68	2.21	0.29	0.18	0.31
OMC-1	25.66	10.31	12.11	0.83	0.04	6.45
RMUG-L	24.78	29.27	24.24	0.50	2.14	38.72
RMUG-S	49.78	15.91	39.58	0.32	4.18	21.92
TU-OM-1	3.78	4.50	2.91	0.67	0.02	0.65

5FU, 5-fluorouracil; CDDP, cisplatin; L-OHP, oxaliplatin; PTX, paclitaxel; SN38, 7-ethyl-10-hydroxycamptothecin; VP-16, etoposide.

Table 2. Calculated assay AUC at IC_{50} and clinically achievable AUC for each drug

Cell lines	Calculated assay AUC (μM/h)					
	CDDP	5FU	L-OHP	PTX	SN38	VP-16
MN-1	303.2	49.1†	62.7†	21.0	13.1	14.9†
OMC-1	637.2	742.3	343.9	60.1	3.0	314.9
RMUG-L	615.4	2107.4	688.4	36.6	154.1	1888.9
RMUG-S	1236.4	1144.8	1124.0	23.3	301.1	1069.3
TU-OM-1	94.1	324.3†	82.6†	48.6	1.2†	31.7†
CA-AUC	85.8	418.2	88.7	19.3	1.5	55.6

†Sensitive.
5FU, 5-fluorouracil; AUC, area under the curve; CA, clinically achievable; CDDP, cisplatin; L-OHP, oxaliplatin; PTX, paclitaxel; SN38, 7-ethyl-10-hydroxycamptothecin; VP-16, etoposide.

Ovarian cancer xenograft model. The present study was carried out at the Laboratory Animal Research Center under the control of the animal research committee, in accordance with the Guidelines for Animal Experimentation in the Faculty of Medicine, Tottori University, Yonago, Japan. For these experiments, TU-OM-1 cells in log-phase growth were trypsinized, washed twice with PBS and centrifuged. Viable cells were counted, then 2×10^6 viable cells (in 0.5 mL PBS) were injected under aseptic conditions into the peritoneal cavities of female nude mice. Mice were then assigned randomly to one of four groups (five mice per group) and treatment was started five days later as follows. Group 1, intraperitoneal (i.p.) PBS weekly for 3 weeks; group 2, i.p. L-OHP weekly (12.5 mg/kg per injection) for 3 weeks; group 3, i.p. 5FU weekly (25 mg/kg per injection) for 3 weeks; and group 4, i.p. L-OHP and with 5FU weekly for 3 weeks.

Statistical analysis. Statistical analyses were carried out using the GraphPad Prism Version 5 program (GraphPad Software, San Diego, CA, USA). Data are presented as mean ± SD. Survival distributions were calculated using the Kaplan–Meier method, and the significance of apparent differences in survival distribution between groups was tested with log-rank tests. $P < 0.05$ was considered statistically significant.

Results

Sensitivity to anticancer agents. The IC_{50} values of each cell line for anticancer agents are shown in Table 1. The IC_{50}s ranged from 3.78 to 49.78 μM for CDDP, from 0.68 to 29.27 μM for 5FU, from 2.21 to 39.58 μM for L-OHP, from 0.29 to 0.83 μM for PTX, from 0.02 to 4.18 μM for SN38 and from 0.31 to 38.72 μM for VP-16, indicating that these cell lines showed various sensitivities to anticancer agents. When the assay AUC was compared with clinically achievable AUC using a standard dose of each drug, two of five cell lines (MN-1, TU-OM-1) were

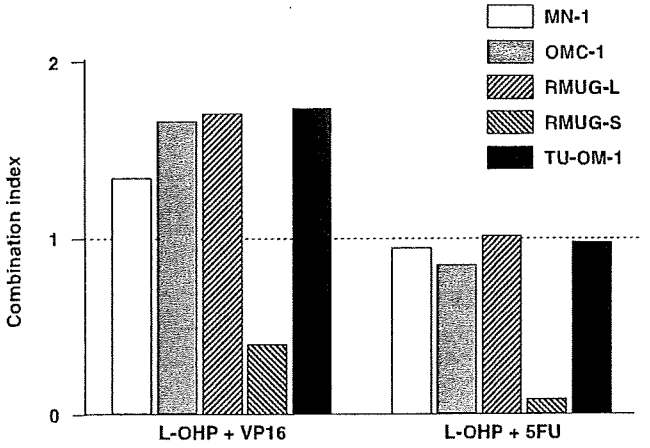

Fig. 1. Effects of 5-fluorouracil (5FU) are synergistic or additive with those of oxaliplatin (L-OHP). L-OHP was combined with 5FU or etoposide (VP-16) at a fixed ratio that spanned the individual IC_{50} of each drug. The combination effects of L-OHP and 5FU were synergistic or additive in all MAC cell lines tested. Data were analyzed by the method of Chou and Talalay[20] to determine the combination index values. The results shown are an average of at least two independent experiments.

defined as sensitive to L-OHP, 5FU or VP-16 and only one (TU-OM-1) was sensitive to SN38. All cell lines were resistant to CDDP and PTX (Table 2).

Combination effects of oxaliplatin and 5-fluorouracil or etoposide. We then examined cell proliferation after treating with both L-OHP and 5FU or VP-16, because these drugs were effective for two of five MAC cell lines. When L-OHP was combined with 5FU, the CI values at an effective dose of 50 (effective dose means the percentage of inhibition of cell growth using the drug combinations in the experiment) were less than 1.1 for all of the five MAC cell lines. The CI values were less than 1.1 for only one cell line when L-OHP was combined with VP-16 (Fig. 1). Thus, combination treatment of L-OHP with 5FU might have more cytotoxic effect on MAC cells than that with VP-16.

Next, to test whether combining L-OHP with 5FU would suppress cell growth at the concentrations calculated by clinically achievable AUC, we treated MAC cell lines with 3.1 μM L-OHP and 5.8 μM 5FU. Seventy-two hours after exposure, growth inhibition was measured using the MTT assay. When L-OHP was combined with 5FU, more than 50% of cell growth suppression was observed in four of five MAC cell lines (Fig. 2a). The number of Annexin V-positive cells also increased additively 24 h after treating with L-OHP and 5FU in all cell lines (Fig. 2b,c).

Oxaliplatin combined with 5-fluorouracil on survival in a mouse xenograft model. After confirming that L-OHP combined with

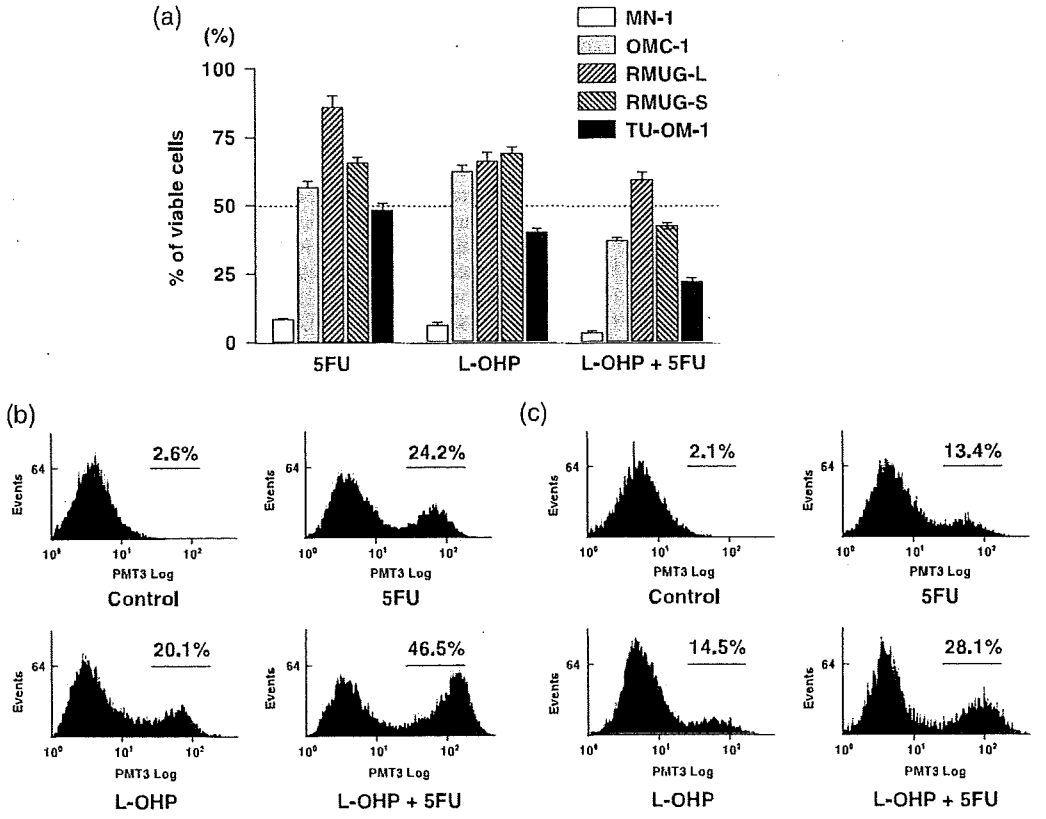

Fig. 2. The effects of oxaliplatin (L-OHP) combined with 5-fluorouracil (5FU) on mucinous adenocarcinoma (MAC) cell proliferation and apoptosis. (a) Five MAC cell lines were treated with 3.1 μM (L-OHP) and/or 5.8 μM 5FU for 72 h compared with the control (phosphate-buffered saline). Cell-growth inhibition was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-dyphenyltetrazolium bromide assay. Cell growth was suppressed more than 50% in four of five cell lines when L-OHP was combined with 5FU at the same concentrations calculated by clinically achievable AUC. Points represent mean ± SD from six dishes. Treatment with L-OHP combined with 5FU increased apoptotic cells. Cells were treated with 3.1 μM L-OHP in the presence or absence of 5.8 μM 5FU for 24 h, then stained with Annexin V-PE. The number of apoptotic cells increased additively after treating with L-OHP combined with 5FU in (b) MN-1 cells and (c) TU-OM-1 cells. Similar results were obtained in the other three cell lines (data not shown). The results shown represent duplicate experiments.

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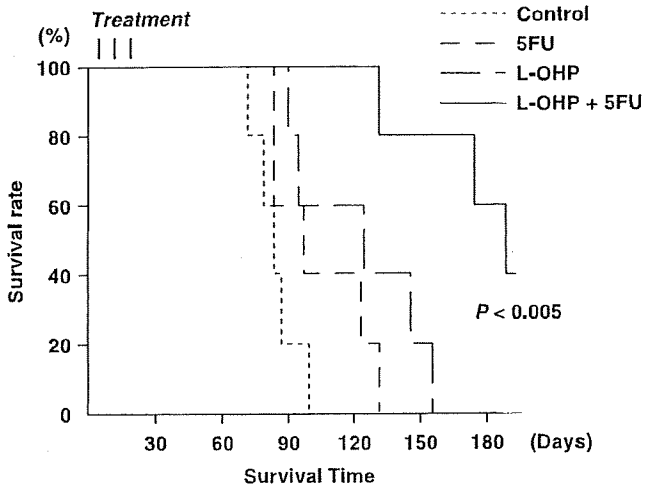

Fig. 3. Treatment with oxaliplatin (L-OHP) combined with 5-fluorouracil (5FU) prolongs survival in mice with implanted TU-OM-1 cells. Female nude mice (five per group) were given an intraperitoneal (i.p.) injection of 2×10^6 TU-OM-1 cells followed by weekly i.p. injections of 250 μL PBS, 12.5 mg/kg L-OHP, and/or 25 mg/kg 5FU for 3 weeks (days 5, 12, 19). Treatment with L-OHP and 5FU prolonged survival relative to treatment with PBS, L-OHP or 5FU ($P < 0.005$).

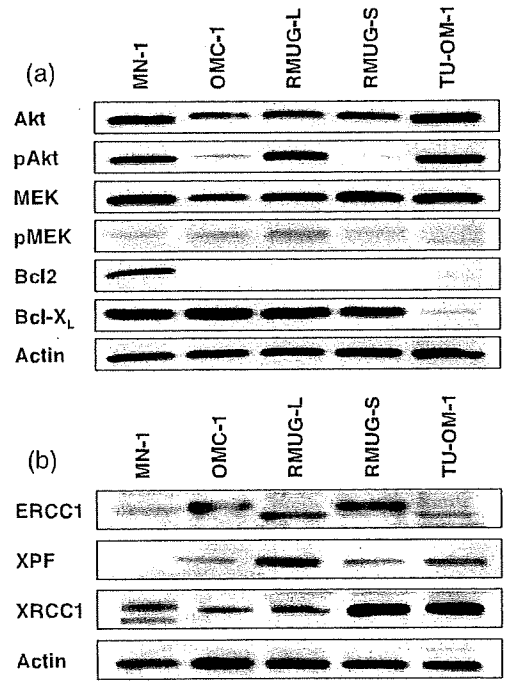

Fig. 4. The high protein expression levels of ERCC1 in oxaliplatin-resistant mucinous adenocarcinoma (MAC) cells. (a) Protein expression of the cell-survival signaling pathways (Akt, MEK, Bcl2, and Bcl-X_L) and (b) DNA repair (ERCC1, XPF, and XRCC1) in MAC cells was determined by Western blot analysis. The results shown represent duplicate experiments.

5FU reduced cell viability and enhanced apoptosis *in vitro*, we examined the effect of combination treatment of L-OHP and 5FU on survival in a xenograft model of MAC. Mice treated with both L-OHP and 5FU survived significantly longer than those treated with PBS, 5FU or L-OHP ($P < 0.005$) (Fig. 3). The median survival times were 188 days for L-OHP with 5FU treatment, 83 days for PBS treatment, 96 days for 5FU treatment

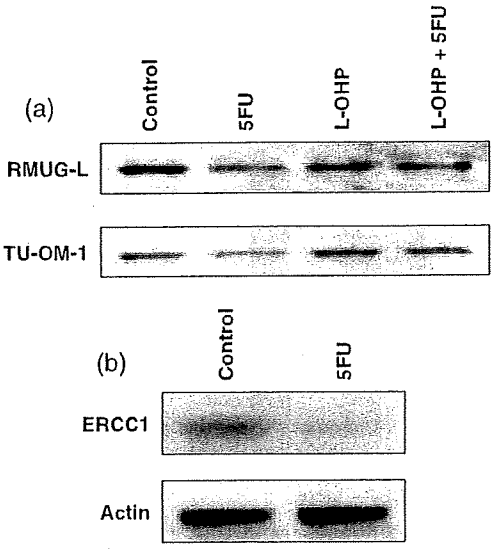

Fig. 5. 5-fluolouracil (5FU) down-regulates ERCC1 expression. (a) Each of the cell lines was treated by 5FU with or without oxaliplatin (L-OHP) for 24 h. The cells were then collected and protein expressions of ERCC1 were determined by Western blot analysis. After treatment with 5FU, the protein expression levels of ERCC1 were down-regulated. The results shown represent duplicate experiments. (b) Nude mice bearing TU-OM-1 were treated with phosphate-buffered saline (PBS) or 5FU intrapenitoneally for 24 h. Then, the expression levels of ERCC1 protein were determined by Western blot analysis. ERCC1 proteins were down-regulated only in tumors from mice treated with 5FU. The results shown represent duplicate experiments.

and 124 days for L-OHP treatment. This finding indicates that L-OHP combined with 5FU resulted in prolonged survival in nude mice bearing TU-OM-1 cells.

Protein expression of cell-survival and DNA repair pathways. The protein expression levels of Akt, phosphorylated (p) Akt, MEK, pMEK, Bcl2 and Bcl-X_L were not related to the sensitivity of anticancer agents tested (Fig. 4a). Among DNA repair proteins, higher ERCC1 expression was observed in L-OHP resistant cells (OMC-1, RMUG-L, RMUG-S) (Fig. 4b).

Next, we examined the protein expression level of ERCC1 at 24 h after treatment with L-OHP and/or 5FU. ERCC1 expression was suppressed after treating with 5FU with or without L-OHP in RMUG-L and TU-OM-1 cells (Fig. 5a). Similar results were obtained in the other three cell lines. Furthermore, Western blot analysis of tumor tissues verified that 5FU down-regulated ERCC1 protein expression in tumor cells (Fig. 5b).

Discussion

Determining cellular sensitivity is difficult due to the wide range of IC_{50} to anticancer agents. Additionally, protein binding of drug and drug stability in the culture medium are important pharmacodynamic factors. Assay AUC is a useful method to predict cellular sensitivity to anticancer agents, which correlates to clinical response.[24,25] In the present study, we calculated assay AUC to assess drug sensitivity and found that two (MN-1, TU-OM-1) of five cell lines were sensitive to VP-16, 5FU and L-OHP, and one cell line (TU-OM-1) to SN38, whereas all cell lines were resistant to CDDP and PTX, which have been used as a standard chemotherapy for EOC. Furthermore, combination treatment of L-OHP with 5FU has more cytotoxic effect on MAC cells than that of L-OHP with VP-16 and suppressed cell proliferation more than 50% in four of five cell lines at the same concentrations calculated by clinically achievable AUC. We confirmed the combination effect of L-OHP and 5FU in a MAC

xenograft model. Combining L-OHP and 5FU prolonged the survival of these mice compared with those treated with L-OHP or 5FU alone. These data provide clear evidence that this combination therapy may be effective for MAC. To our knowledge, this is the first study to show that the combination of L-OHP and 5FU is effective for MAC both *in vitro* and *in vivo*.

L-OHP is a third-generation platinum compound with a spectrum of activity and toxicity that differs from CDDP by the presence of a DACH ligand, although L-OHP and CDDP act theoretically by the same mechanism of action.[26,27] Like other DACH platinum compounds, L-OHP has different sensitivity profiles in a broad range of cancer cell lines, and has shown activity in a number of cell lines, including ovarian cancer, that exhibit intrinsic or acquired resistance to CDDP.[11–13] We also found two of five MAC cell lines to be sensitive to L-OHP, although all cell lines were resistant to CDDP. Several explanations as to the mechanisms for this lack of cross-resistance have been proposed. In contrast to CDDP, cellular accumulation of L-OHP seems to be less dependent on copper transporter 1 (CTR1), which is the major copper influx transporter;[28] a lower intracellular drug concentration and fewer platinum (Pt)–DNA adducts are sufficient for L-OHP to exert its cytotoxicity;[29] and mismatch repair protein does not bind to DACH–Pt–DNA adducts.[30]

Recently, several reports revealed the importance of the nucleotide excision repair (NER) process in the sensitivity to L-OHP and CDDP.[31,32] Furthermore, excision repair cross-complementing rodent repair deficiency, complementation group 1 (ERCC1) is essential for NER process, and its mRNA or protein expression levels have been involved in L-OHP sensitivity.[31–33] Indeed, we observed high protein expression levels of ERCC1 in L-OHP-resistant cell lines. These results suggest that ERCC1 protein expression level may be related to L-OHP sensitivity in MAC cells.

In this study, the combination of L-OHP and 5FU revealed synergistic or additive effects in all MAC cell lines.[20] We next attempted to elucidate the reason why this combination of L-OHP and 5FU contributes to the increasingly cytotoxic effect for MAC cells, and found that the protein expression levels of ERCC1 were down-regulated after treatment of 5FU both *in vitro* and *in vivo*. Ojima *et al.*[34] reported that 5FU post-treatment gradually inhibited mRNA expression of ERCC1 in colon cancer cell lines. Suppression of the protein expression of ERCC1 by 5FU may affect recombination DNA repair efficiency. Therefore, these results suggest that the down-regulation of ERCC1 protein expression by treating with 5FU may enhance the cytotoxic effects of L-OHP.

We found that the combination treatment of L-OHP and 5FU had marked cytotoxic effects on MAC cells, even on cell types known to be resistant to conventional platinum- and taxane-based chemotherapy. We also found that ERCC1 expression levels probably affected the cytotoxic effects of L-OHP, and this effectiveness may be related to down-regulation of ERCC1 protein expression by 5FU exposure. Therefore, we concluded that this combination treatment is worth exploring as a treatment modality for MAC. Our next step is to evaluate the ability of ERCC1 expression levels to predict response to chemotherapy in clinical studies. Currently, a phase III clinical trial of L-OHP and capecitabine, which is a prodrug of 5FU, *versus* carboplatin and PTX in MAC, is underway at the Gynecologic Oncology Group (GOG). We hope that this combination therapy will prolong the survival of patients with advanced MAC.

Acknowledgments

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Clinicopathological characteristics of mucinous adenocarcinoma of the ovary

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ABSTRACT

Objective. We conducted the present study to clarify the clinicopathological characteristics of mucinous adenocarcinoma.

Methods. Two hundred twenty-five patients were diagnosed with mucinous adenocarcinoma at individual institutes and underwent primary treatment between 1998 and 2003. Of these patients, 189 patients who could undergo central pathological review were enrolled in this study. Of 189 patients undergoing central pathological review, 64 patients (33.9%) were diagnosed with mucinous invasive adenocarcinoma, 45 mucinous intraepithelial carcinoma, and 42 mucinous tumor of borderline malignancy. Twenty-five patients were diagnosed with other histological subtypes, including 8 endometrioid adenocarcinoma, 5 clear cell carcinoma, 3 serous adenocarcinoma, and 4 mixed type. There were 13 cases of metastatic mucinous adenocarcinoma, including 7 pseudomyxoma peritonei. Four hundred thirty-three patients with serous adenocarcinoma were used as controls.

Results. Forty-five patients with mucinous invasive carcinoma were in FIGO I–II stages and 19 in III–IV stages. There was no difference in the outcome between mucinous invasive adenocarcinoma and serous adenocarcinoma in I–II stage patients and III–IV stage patients with optimal operation. In contrast, patients with mucinous invasive adenocarcinoma receiving suboptimal operation showed a significantly worse prognosis (survival rate: 27.8% vs. 61.5%). The response rate to chemotherapy for mucinous invasive adenocarcinoma was significantly lower than for serous adenocarcinoma (12.5% vs. 67.7%).

Conclusions. The diagnosis of mucinous invasive adenocarcinoma was difficult. Since patients with mucinous invasive adenocarcinoma had a lower response to chemotherapy, aggressive cytoreductive surgery was an effective treatment to improve the prognosis for advanced stage patients. A new chemotherapeutic regimen should be established for mucinous adenocarcinoma of the ovary.

Introduction

The current standard treatment for epithelial ovarian cancer is cytoreductive surgery followed by combination chemotherapy with carboplatin and paclitaxel [1]. Combination chemotherapy shows a good response to epithelial ovarian cancer and the response rate is 70–80% [2,3]; however, most clinical trials reflect the results of serous adenocarcinoma of the ovary, which comprises the majority of epithelial ovarian cancer. Tumor histology is an independent prognostic factor in patients with ovarian cancer [4]. Patients with clear cell carcinoma or mucinous adenocarcinoma of the ovary showed a significantly worse prognosis in the retrospective review of several Gynecologic Oncology Group (GOG) trials. Additionally, several authors indicated that the histological subtype of epithelial ovarian cancer strongly related to the chemo-response [5,6]. Hess et al. reported that patients with advanced mucinous adenocarcinoma have a poorer response to platinum-based chemotherapy compared with patients with other histologic subtypes of epithelial ovarian cancer [6]. However, there is very little prospective data to guide surgical or chemotherapy management. It is therefore an important issue to determine the optimal regimen based on histological subtype. Now, an international cooperative phase III study on clear cell carcinoma of the ovary is ongoing as the Japanese

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Gynecologic Oncology Group and Gynecologic Cancer Intergroup study.

Mucinous ovarian cancer is the third most common type, and the incidence of this tumor is approximately 10% of all primary epithelial ovarian cancer [7]; however, the clinicopathological characteristics have not been clarified. With careful exclusion of non-invasive, and metastatic mucinous tumors, pure mucinous adenocarcinoma primary of the ovary appears to be substantially less common than in previous reports [8]. Accordingly, a central pathological review with definitive criteria is necessary to clarify the substantive clinical behavior of mucinous adenocarcinoma of the ovary.

We conducted multicenter retrospective analysis in the central pathological review to clarify the clinicopathological characteristics of mucinous adenocarcinoma. To our knowledge, the current study is one of the largest numbers of mucinous adenocarcinoma reviewed by central pathologist review.

Materials and methods

Between 1998 and 2003, 1400 patients with International Federation of Gynecology and Obstetrics (FIGO) stages I to IV epithelial ovarian cancer underwent primary treatment at Jikei University Kashiwa Hospital, Jichi Medical School, National Shikoku Cancer Center, National Hospital Organization Osaka National Hospital, Hyogo Cancer Center, Iwate Medical University, Mie University, Nara Prefectural Nara Hospital, Shiga University of Medical Science, National Defense Medical College, Saga University, Kawasaki Medical College, and Tottori University. There were 655 patients (46.8%) with serous adenocarcinoma, 246 patients (17.6%) with endometrioid adenocarcinoma, 274 patients (19.6%) with clear cell adenocarcinoma, and 225 patients (16.1%) with mucinous adenocarcinoma by pathological diagnosis at each institution. Two hundred twenty-five patients were initially diagnosed with primary invasive ovarian mucinous adenocarcinoma at individual institutions. Of these 225 patients, 189 patients, whose specimens could undergo central pathological review, were enrolled in this study. Thirty-six patients refused consent for the central pathological review. Medical records were available for all 189 patients. All patients underwent primary cytoreductive surgery. In advanced stage patients, optimal surgery was performed in 10 patients with mucinous adenocarcinoma and 174 with serous adenocarcinoma. Nine patients with mucinous adenocarcinoma and 168 with serous adenocarcinoma could not undergo optimal operation. All patients in both mucinous adenocarcinoma and serous adenocarcinoma received platinum based chemotherapy.

All specimens were reviewed according to the diagnostic criteria of the central pathological review [9–11]. Briefly, mucinous intraepithelial adenocarcinoma is defined as a non-invasive tumor with severe cytological atypia regardless of the degree of stratification and a complex intracystic growth pattern. Microinvasion is defined as a tumor with invasive foci up to 5 mm of greatest linear measurement in any single focus. Invasion beyond 5 mm warrants a diagnosis of invasive mucinous adenocarcinoma. Microinvasive mucinous adenocarcinoma is defined as mucinous intraepithelial adenocarcinoma with microinvasion. Metastatic adenocarcinoma is defined as a tumor with consistent morphological features such as nodular involvement and infiltrative invasion, and synchronous/asynchronous extraovarian mucinous adenocarcinoma. Representative cases are shown in Fig. 1. We did not evaluate grade of mucinous adenocarcinoma in our series, because histological grading is not included in the World Health Organization (WHO) criteria for mucinous adenocarcinoma of the ovary. Four hundred thirty-three patients with serous adenocarcinoma, whose medical records were available, were used as controls in our series.

For patients with measurable lesions, response to chemotherapy was evaluated with computed tomography. Response was defined as follows: complete response (CR) was defined as the complete disappearance of all measurable lesions, partial response (PR) was defined as a 50% or more decrease in the sum of the products of the largest diameter and perpendicular diameter of the tumor, no change (NC) meant a steady state or a response less than PR, and progressive disease (PD) was defined as an unequivocal increase of at least 25% in tumor size and/or the appearance of new lesions. CR and PR were defined as responders.

Patient survival distribution was calculated using the Kaplan–Meier method. The significance of the survival distribution in each group was tested by the log-rank test. A value of $p<0.05$ was considered significant.

Results

Under the central pathological review, 151 patients were reclassified into mucinous tumor of the ovary (Table 1). Twenty-five patients were diagnosed with other histological subtypes, including 8 with endometrioid adenocarcinoma, 5 with clear cell carcinoma, 3 with serous adenocarcinoma, and 4 with mixed type. There were 13 cases of metastatic mucinous adenocarcinoma, including 7 pseudomyxoma peritonei.

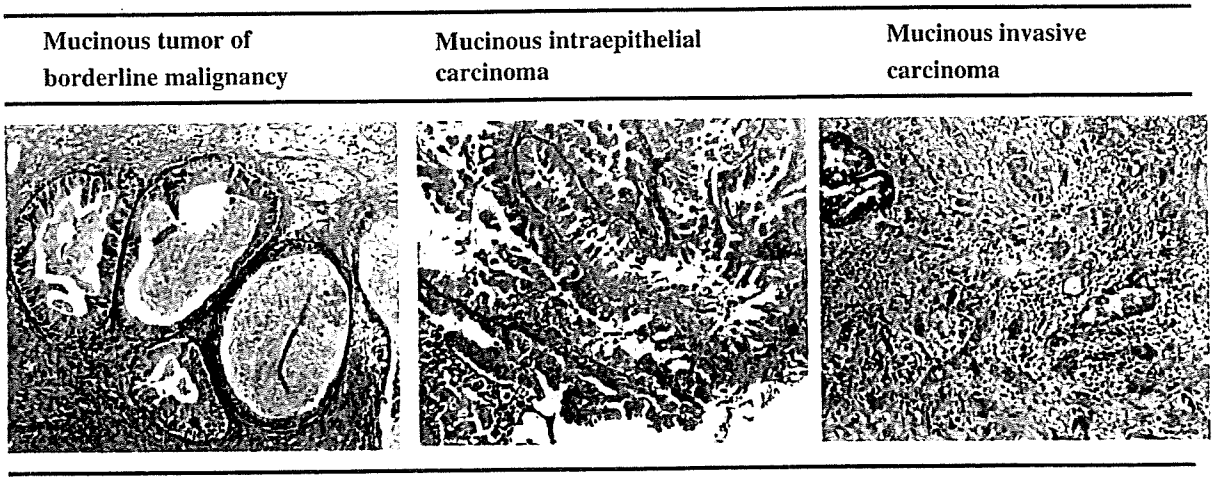

Fig. 1. Representative case of mucinous tumor. Mucinous tumor of borderline malignancy. Mucinous intraepithelial carcinoma. Mucinous invasive carcinoma.

Table 1
Results of the central pathological review

Diagnosis	*Number*
Mucinous tumor	151
Mucinous invasive adenocarcinoma	64
Mucinous intraepithelial carcinoma	45
Mucinous tumor of borderline malignancy	42
Metastatic mucinous carcinoma	13
Others	25
Total	189

Forty-five patients with mucinous invasive carcinoma were in FIGO stages I–II (Table 2). The incidence of patients with mucinous invasive adenocarcinoma in FIGO stages III–IV has a tendency to be higher than mucinous tumor of borderline malignancy or mucinous intraepithelial adenocarcinoma.

No patients with mucinous intraepithelial carcinoma died. The 3-year survival rate was 87.9% for mucinous tumor of borderline malignancy and 83.8% for mucinous invasive adenocarcinoma. In patients with mucinous invasive adenocarcinoma, the 3-year survival rate was 90.0% for stage Ia–b, 94.1% for stage Ic, 100% for stage II, 56.9% for stage III, and 66.7% for stage IV. The 3-year survival rate was 100% for stage Ia–b, 91.6% for stage Ic, 86.8% for stage II, 69.3% for stage III, and 35.3% for stage IV in patients with serous adenocarcinoma. There was no significant difference in the outcome between mucinous invasive adenocarcinoma and serous adenocarcinoma in FIGO I–II stage patients. Furthermore, the outcome did not differ between mucinous and serous adenocarcinoma in III–IV stage patients with optimal operation defined as residual tumor less than 1 cm (Fig. 2). In contrast, nine patients with mucinous invasive adenocarcinoma who could not receive the optimal operation had significantly worse prognosis than 168 patients with serous adenocarcinoma (survival rate: 27.8% vs. 61.5%, $p = 0.013$).

The response rate to chemotherapy for mucinous invasive adenocarcinoma was significantly lower than that for serous adenocarcinoma (Table 3). Of 24 mucinous patients with measurable lesions, only 3 responded to adjuvant chemotherapy. A patient showing CR was a stage III patient with mucinous intraepithelial carcinoma whose residual disease was 5 cm of diameter in upper abdomen. Two stage III patients with mucinous invasive carcinoma showed PR whose residual diseases (3–5 cm) were in upper abdomen.

Discussion

This study indicated two important findings for the treatment of patients with mucinous adenocarcinoma of the ovary. First, the diagnosis of primary mucinous invasive adenocarcinoma of the ovary is difficult. Second, complete surgical resection might be critical to improve the survival of patients with mucinous adenocarcinoma, because of its low chemosensitivity.

Although mucinous adenocarcinoma comprises about 10% of primary ovarian cancer, its incidence is controversial. Hart, WR demonstrated a high index of suspicion that a mucinous tumor was actually a metastasis from another organ [12]. Accordingly, mucinous tumors in the ovary should be distinguished from metastatic ovarian involvement from other primary sites, including the lower gastrointestinal

Table 2
FIGO stage in patients with mucinous tumor of the ovary

FIGO stage	Invasive carcinoma	Intraepithelial carcinoma	Borderline malignancy
Ia–b	18	21	18
Ic	22	20	15
II	5	0	1
III	16	3	7
IV	3	1	1

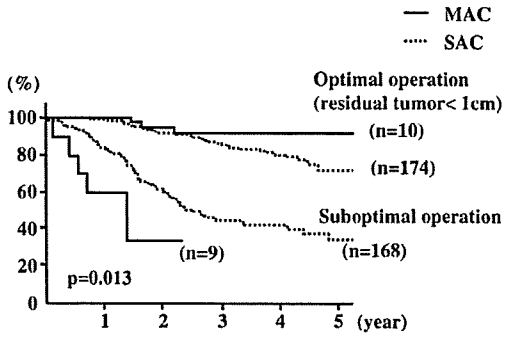

Fig. 2. The survival rate and surgical completeness in advanced-stage (FIGO stages III–IV) patients with mucinous and serous adenocarcinoma. In patients with optimal operation, there is no significant difference in survival between mucinous invasive adenocarcinoma and serous adenocarcinoma. In contrast, patients with mucinous invasive adenocarcinoma who could not undergo an optimal operation showed significantly worse prognosis than those with serous adenocarcinoma (survival rate: 27.8% vs. 61.5%, $p = 0.013$).

tract, appendix, stomach, and pancreas. In the literature, 40 cases (77%) were metastatic and 12 cases (23%) were primary among 52 cases of mucinous carcinoma of the ovaries [8]. On the other hand, we found only 13 patients (6.9%) with metastatic mucinous adenocarcinoma. Since the majority of patients with epithelial ovarian cancer underwent a thorough inspection of digestive organs in our series, the incidence of metastatic mucinous adenocarcinoma might be lower than expected. Consequently, clinical examination of digestive organs, such as the gastrointestinal tract, stomach, and pancreas, may be necessary before surgery.

Mucinous adenocarcinoma is defined as showing obvious stromal invasion in WHO classification of 1973 [13]; however, the evaluation of stromal invasion, especially expansile invasion, is difficult in mucinous tumors. In the literature, there were only three pure mucinous carcinomas (2.4%) among 124 consecutive ovarian carcinomas with uniform criteria to exclude microinvasive or intraepithelial mucinous tumors [8]. Forty-two cases of 151 mucinous tumors were diagnosed as mucinous tumor of borderline malignancy, 45 cases of mucinous intraepithelial carcinoma, and 64 cases of mucinous invasive adenocarcinoma under the central pathological review. Our findings supported the report of Seidman that primary mucinous invasive adenocarcinoma of the ovary appears to be substantially less common than in previous reports [7].

Because most patients with mucinous adenocarcinoma are in the early stage [14], these patients showed a better prognosis than those with other histological types [15]. It is known that the majority of mucinous adenocarcinoma is either well- or moderately differentiated and this contributes to the low risk of relapse for FIGO stage I tumors [15]. We also found that patients in the early stage showed a good outcome. On the other hand, patients with advanced mucinous adenocarcinoma had a poorer outcome which is well recognized, supporting the findings of Winter et al. [4]. There was no significant difference in survival between mucinous invasive adenocarcinoma and serous adenocarcinoma in patients with optimal operation. In contrast, patients with suboptimal operation showed a significantly worse prognosis than those with serous adenocarcinoma. This is the first study to demonstrate the relationship between residual tumor and prognosis in advanced-stage patients with mucinous invasive

Table 3
Response to chemotherapy of mucinous and serous adenocarcinoma of the ovary

	CR	PR	NC	PD	Response rate
Mucinous ($n = 24$)	1	2	7	14	12.5%
Serous ($n = 189$)	54	74	34	27	67.7%

adenocarcinoma. The amount of residual tumor after primary cytoreductive surgery and sensitivity to chemotherapy are absolutely independent prognostic factors in patients with advanced epithelial ovarian cancer [4,16].

In our series, mucinous invasive adenocarcinoma showed a low response to chemotherapy (12.5%), whereas 67.7% with serous adenocarcinoma responded to platinum-based chemotherapy. Several authors indicated that patients with mucinous adenocarcinoma showed a lower response to platinum-based chemotherapy [6,17,18]. The case-controlled study by Hess et al. concluded that patients with advanced mucinous adenocarcinoma had a poorer response to platinum-based chemotherapy than patients with other histological subtypes of epithelial ovarian cancer [6]. The Hellenic Cooperative Oncology Group trial revealed that the overall response rate was 38.5% for mucinous adenocarcinoma and 70% for serous adenocarcinoma [17]. The retrospective analysis of 21 patients reviewed the response to first-line chemotherapy of patients with mucinous adenocarcinoma, and eight patients (42%) responded to first-line platinum-based chemotherapy [18]. These findings suggested that low chemosensitivity might affect the poor outcome of mucinous adenocarcinoma. Currently, the optimal chemotherapy regimen for patients with mucinous adenocarcinoma has not been established, so aggressive cytoreductive surgery is the only effective treatment to improve the prognosis of advanced-stage patients with mucinous invasive adenocarcinoma.

Recently, we found that combination chemotherapy of Oxaliplatin and 5FU might be an effective and a pivotal candidate as a novel treatment strategy for mucinous adenocarcinoma [19]. In our study, the combination of Oxaliplatin and 5FU resulted in additive or synergistic effects on mucinous cells. Additionally, this combination significantly prolonged survival in a mucinous adenocarcinoma xenograft model of nude mice. We are planning phase II trial to establish a new chemotherapeutic regimen for mucinous adenocarcinoma of the ovary.

Conflict of interest statement
The authors declare that there are no conflicts of interest.

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Preoperative and Intraoperative Assessments of Depth of Myometrial Invasion in Endometrial Cancer

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Objective: Preoperative and intraoperative assessments of myometrial invasion (MI) are commonly used for planning surgical procedures such as dissection of the para-aortic node; however, the assessments often differ from the final diagnosis determined by pathological examination. The present study evaluated the accuracy of preoperative and intraoperative assessments of MI.

Methods: A total of 191 patients with endometrial cancer, who underwent hysterectomy from 1995 to 2007 in Tottori University Hospital, were included in this study. One hundred seventy-four patients underwent endometrial curettage or Pipelle biopsy preoperatively. Histological grade was compared between preoperation and postoperation. Magnetic resonance imaging (MRI) was performed before surgery, and the depth of MI was assessed as 3 levels (no MI, <50%, and >50%). During surgery, the uterine wall was incised at the most invasive part, and then, intraoperative gross assessment was evaluated as less than or greater than 50%.

Results: Histological evaluation revealed that 34 patients had no invasion, 97 had less than 50% MI, and 60 had greater than 50% MI. On MRI assessment, 135 patients had correct diagnoses, and the accuracy was 70.7%. Regarding the diagnosis of greater than 50% MI depth, the accuracy, the sensitivity, and the specificity of the MRI assessment were 83.2%, 75.0%, and 85.7%, respectively. Seventeen patients were overestimated, and 15 patients were underestimated by the MRI assessment. On intraoperative gross assessment, 162 patients had correct diagnoses, 8 patients were overestimated, and the remaining 21 patients were underestimated. The accuracy of the gross assessment was 84.8%, the sensitivity was 65.0%, and the specificity was 93.9%. The preoperative grading accuracy was 71.8% (125/174). A discrepancy between preoperative and postoperative grades was more frequent in a low-grade tumor. The incidence of underdiagnosis was significantly higher in patients with a grade 3 (G3) tumor than in those with a G1 or G2 tumor in both assessments.

Conclusions: The present study suggests that gross assessment may be useful to determine MI of less than 50%, although patients with a G3 tumor were more frequently underestimated.

Key Words: Endometrial cancer, Diagnosis, Myometrial invasion, MRI, Gross assessment

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Endometrial cancer is the most common gynecologic malignancy in the United States and has increased in Japan.[1,2] Approximately 80% of cases are diagnosed in stage I, and surgery is the primary treatment.[3–5] The International Federation of Gynecology and Obstetrics implemented a surgical staging system for endometrial cancer.[6] The surgical procedure typically consists of hysterectomy, bilateral salpingo-oophorectomy, and retroperitoneal lymphadenectomy, including para-aortic nodes; however, lymphadenectomy, including para-aortic nodes in the surgical management of all patients with endometrial cancer, remains controversial.[7,8]

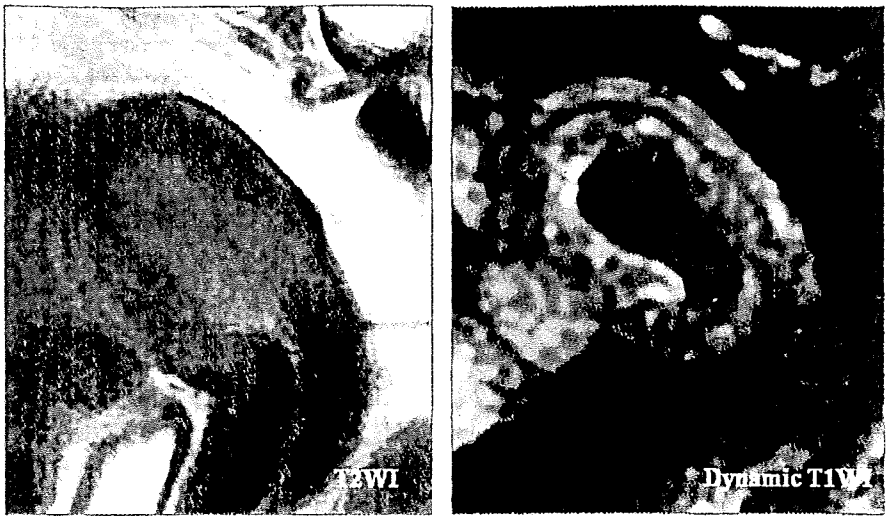

FIGURE 1. Myometrial assessment on MRI. On T2WI study, disruption of the junctional zone was observed. Higher signal intensity penetrated the myometrium but less than 50%. The dynamic T1WI study also showed the disruption of subendometrial enhancement at the postuterine wall.

Final pathologic information is obviously not available when a surgeon decides whether to perform lymphadenectomy, including para-aortic nodes; therefore, many investigators have assessed alternate means of predicting lymph node involvement.[9] The depth of myometrial invasion (MI) is associated with lymph node involvement.[10–12] Accordingly, preoperative and intraoperative assessments of MI are commonly used to plan surgical procedures such as dissection of para-aortic nodes.

Magnetic resonance imaging (MRI) is commonly used to assess MI.[13–16] Generally, gross examination of MI during surgery is also performed[17]; however, these assessments often differ from the final diagnosis determined by pathological findings. The present study evaluated the accuracy of preoperative and intraoperative assessments of MI.

MATERIALS AND METHODS

A total of 191 patients with endometrial cancer, who underwent hysterectomy from 1995 to 2007 in Tottori University Hospital, were included in this study. Age ranged from 33 to 89 years (mean, 57 years). There were 150 postmenopausal women and 41 premenopausal women in our series. There were 33 stage IA, 85 stage IB, 27 stage IC, 2 stage IIA, 3 stage IIB, 8 stage IIIA, 1 stage IIIB, 23 stage IIIC, 1 stage IVA, and 8 stage IVB. One hundred seventy-three patients had endometrioid adenocarcinomas, 8 adenosquamous carcinomas, 3 adenoacanthomas, 3 serous adenocarcinomas, 3 clear cell adenocarcinomas, and 1 mucinous adenocarcinoma.

One hundred seventy-four patients underwent endometrial curettage or Pipelle biopsy preoperatively. Histological grade was compared between preoperation and postoperation in those 174 patients. Magnetic resonance imaging was performed before surgery, and the depth of MI was assessed as 3 levels (no MI, <50%, and >50%). Myometrial invasion was evaluated according to published criteria based on T2-weighted images (T2WI) and dynamic T1-weighted images (T1WI) MRI as follows.[16,18] The recognition of disruption or irregularity of the junctional zone on T2WI was considered as an invasive tumor. In the dynamic study, intact and smooth subendometrial enhancement was considered as a non-infiltrative tumor. Disruption or irregularity of the subendometrial enhancement was indicative of MI. When the signal intensity of the tumor penetrated the outer half of the myometrium, either on T2W1 or dynamic study, greater than 50% MI was diagnosed[19] (Fig. 1). All assessments were carried out by radiologists. Magnetic resonance imaging was performed using a 1.5-T superconducting unit (Vision or Symphony; Siemens, Erlangen, Germany) or 3.0-T unit (Sigma EXCITE HD; GE Medical systems, Milwaukee, Wis) with a phased array coil.

As a rule, the surgical procedure consisted of washing for cytology, hysterectomy, bilateral salpingo-oophorectomy, and pelvic lymphadenectomy. During surgery, the uterine wall was incised at the most invasive part, and then, intraoperative gross assessment was evaluated as less than or greater than 50% MI by gynecologic oncologists. When the MI was judged as greater than 50% with intraoperative gross assessment, para-aortic lymphadenectomy was added. Of 191 patients, 23 did not undergo lymphadenectomy, 125 patients underwent pelvic lymphadenectomy alone, and 43 underwent both pelvic and para-aortic lymphadenectomies.

The accuracy of these assessments was determined according to the final pathological evaluation of the specimen. Accuracy, sensitivity, and specificity were calculated using standard statistical formulas. Statistical analyses were performed using Statview version

TABLE 1. Magnetic resonance imaging assessment

	Histological Diagnosis		
	No Invasion	MI < 50%	MI > 50%
No invasion	27	18	0
MI < 50%	6	63	15
MI > 50%	1	16	45

TABLE 2. Gross assessment

	Histological Diagnosis		
	No Invasion	MI < 50%	MI > 50%
MI < 50%	34	89	21
MI > 50%	0	8	39

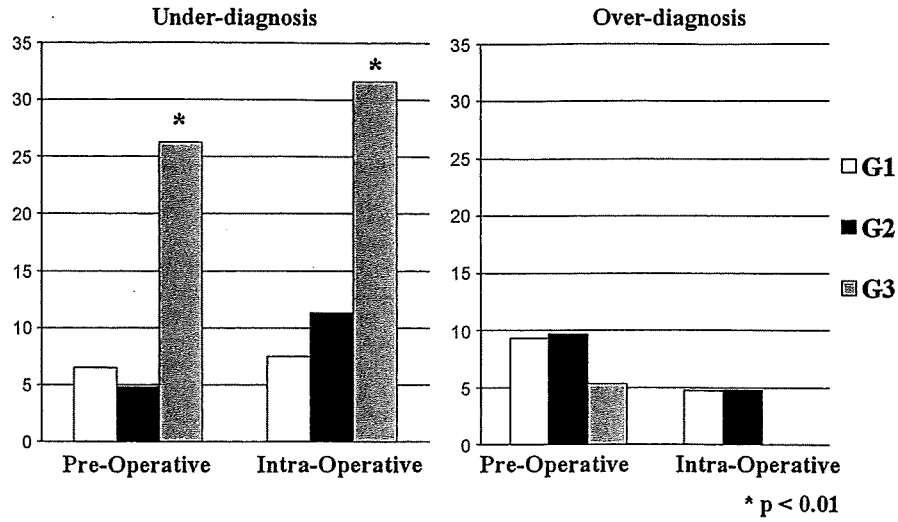

FIGURE 2. Incidences of underdiagnosis and overdiagnosis. The incidence of underdiagnosis for patients with a grade 3 tumor was significantly higher than for those with a G1 or G2 tumor in both preoperative (MRI) and intraoperative (gross) assessments.

5.0-J (Hulinks Inc, Tokyo, Japan). A χ^2 test was used to compare any associations of prognostic factors. $P < 0.05$ was considered statistically significant.

RESULTS

Histological evaluation revealed that 34 had no invasion, 97 had less than 50% MI, and 60 had greater than 50% MI. Lymph node involvement was observed in 28 cases. The incidence of lymph node metastasis for patients with greater than 50% MI was significantly higher (4.6% for <50% MI vs 36.7% for >50% MI, $P < 0.0001$). Of those patients, 16 had pelvic lymph node metastasis alone, and 9 had pelvic and para-aortic lymph node involvement. Independent metastasis to para-aortic lymph nodes was observed in 3 of 43 patients who underwent para-aortic lymphadenectomy. In addition, no patients with less than 50% MI showed recurrence at para-aortic nodes.

The MRI assessment is shown in Table 1. The assessment of MI by MRI did not differ between premenopausal and postmenopausal women. One hundred thirty-five patients had correct diagnoses, and the accuracy of the MRI assessment was 70.7%. Forty-five patients were found to have no invasion by MRI, but histological diagnosis revealed that 18 patients (40%) had less than 50% MI. Regarding the diagnosis of greater than 50% MI depth, the accuracy, the sensitivity, and the specificity of the MRI assessment were 83.2%, 75.0%, and 85.7%, respectively.

On intraoperative gross assessment, 162 patients had correct diagnoses, 8 patients were overestimated, and the remaining 21 patients were underestimated (Table 2). The accuracy of the gross assessment was 84.8%, the sensitivity was 65.0%, and the specificity was 93.9%.

The preoperative grading accuracy was 71.8% (125/174). A discrepancy between preoperative and postoperative grades was more frequent in a low-grade tumor (Table 3).

The incidences of overdiagnosis and underdiagnosis were 13.0% and 25.0% for MRI assessment and 6.1% and 35.0% for gross assessment, respectively. There was no significant difference in the incidences of overdiagnosis and underdiagnosis between MRI and gross assessment.

Three clear cell carcinomas were excluded from grading. Of 188, 107 were diagnosed as grade 1 (56.0%), 62 as grade 2 (32.5%), and 19 as grade 3 (9.9%). Patients with grade 3 showed a significantly higher incidence of underdiagnosis than those with G1 or G2 in both assessments (Fig. 2).

TABLE 3. Histological grades in preoperative and postoperative examinations

	Postoperative (%)		
Preoperative	Grade 1	Grade 2	Grade 3
Grade 1 (n = 111)	84 (75.7)	20 (18.0)	7 (6.3)
Grade 2 (n = 54)	15 (27.8)	35 (64.8)	4 (7.4)
Grade 3 (n = 9)	3 (33.3)	0 (0.0)	6 (66.7)

DISCUSSION

Although the International Federation of Gynecology and Obstetrics recommended in 1988 that adequate surgical staging requires a total abdominal hysterectomy, bilateral salpingo-oophorectomy, and pelvic and para-aortic lymphadenectomies,[6] there is no consensus whether routine pelvic and para-aortic lymphadenectomies are essential for patients with endometrial cancer.[7,8] On the basis of detailed outcome analyses of 915 patients treated at the Mayo Clinic, lymphadenectomy could be omitted if there was no disease beyond the corpus and if the disease was grade 1 or 2, the MI was less than 50%, and primary tumor diameter was 2 cm or less.[20,21] In our series, the incidence of lymph node metastasis in patients with greater than 50% MI was significantly higher. We also performed aortic lymphadenectomy for patients with any histological grade who had greater than 50% MI. Forty-three of 166 patients underwent pelvic or para-aortic lymphadenectomy, and 6.9% of the patients had para-aortic node involvement. In contrast, no patients with less than 50% MI showed recurrence at the para-aortic node. This finding suggests that our criteria that patients judged as having greater than 50% MI should undergo para-aortic lymphadenectomy might be reliable.

Magnetic resonance imaging is most commonly used for preoperative assessment of MI. The accuracy of assessing the depth of MI with dynamic contrast-enhanced MRI ranged from 83.0% to

92.6%.[14] Determining the depth of MI has drawbacks such as polypoid tumor, distension of the endometrial cavity by pyometra, and the presence of leiomyoma.[16,23] In addition, consideration of the menopausal status and selection of the proper pulse sequence affect accurate staging with MRI.[3] In contrast, the assessment of MI did not differ between the premenopausal and the postmenopausal statuses in our series. Regarding the diagnosis of greater than 50% depth of MI, the accuracy of our series was 83.2%. The sensitivity and the specificity of MRI assessment were 75.0% and 85.7%, respectively. Although transvaginal sonography is convenient, MRI provides higher accuracy than other imaging modalities, including transvaginal sonography and computed tomography.[13–15]

On the other hand, the cost of MRI examination is expensive. The accuracy of gross assessment was 84.8%, the sensitivity was 65.0%, and the specificity was 93.9%. In addition, intraoperative gross assessment is a cost-effective method. Gross assessment might be more useful to determine greater than 50% MI than MRI. Pathological examination using frozen sections obtained during the operation improved the level of accuracy, but the procedure is not convenient.[24,25] The accuracy of MRI was equivalent to that of gross assessment in MI. In addition, MRI might be more valuable because it could have other information such as cervical invasion, adnexa, nodal involvements, and free fluid.

We found that both preoperative evaluation by MRI and intraoperative gross assessment tended to underestimate the depth of MI. In particular, the incidence of underdiagnosis was significantly higher in patients with a G3 tumor in both assessments. The infiltration pattern of a G3 tumor might differ from other tumor grades. A G3 tumor is defined as being a high risk of endometrial cancer; therefore, para-aortic dissection should be added for patients with a G3 tumor, regardless of MI.

Of 30% of patients in grade 2, 3 showed a discrepancy between preoperative and postoperative grades. Some authors showed that the discrepancy of Pipelle biopsy was 3% to 50%, and that of dilatation and curettage was 1% to 19%.[26,27] Thus, it is difficult to select high-risk cases with preoperative examination.

Whereas it is important to know the accuracy of preoperative and intraoperative assessments of MI, the present study suggests that gross assessment may be useful to determine greater than 50% MI.

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