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Preclinical report

In vitro sensitivity of human endometrial cancer cell lines to paclitaxel or irinotecan (CPT-11) in combination with other aniticancer drugs

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We have evaluated the growth inhibitory effects of paclitaxel alone or irinotecan (CPT-11) alone and their combined effects with other drugs on human endometrial cancer cell lines. IC_{50} doses of paclitaxel (Tx), SN-38 (active metabolite of CPT-11; 7-ethyl-10-hydroxycamptothecin) and cisplatin, including other drugs which have been used for treatment of patients with endometrial cancer, were examined using five human endometrial cancer cell lines (Ishikawa, HEC-1A, HEC-50B, HEC-59 and HEC-108). When *in vitro* sensitivity was defined IC_{50} lower than 10% of the peak plasma concentration (PPC), all endometrial cancer cell lines were sensitive to paclitaxel and three of five endometrial cancer cell lines were sensitive to SN-38, whereas cisplatin was not active against any endometrial cancer cell lines used in this study. Regarding the other drugs, aclarubicin (ACR) and actinomycin D (ACD) were active against four of five endometrial cancer cell lines, etoposide (VP-16) and pirarubicin (THP) against two, and 5-fluorouracil (5-FU) against only one, while ifosfamide (4-OHIFO) was not active against any endometrial cancer cell lines. When combined effects of paclitaxel or SN-38 with other one drug were determined by the median-effect analysis, paclitaxel followed by cisplatin resulted in synergistic effects to all endometrial cancer cell lines. Paclitaxel followed by SN-38 also had synergistic effects to four cell lines. Sequential but not simultaneous administration of taxol and THP-adriamycin showed synergistic effects to three cell lines. In combinations of SN-38 with other drugs, simultaneous administration of SN-38 and cisplatin resulted in synergistic effects to all cell lines. It is noteworthy that ACD followed by SN-38 showed synergistic effects to all cell lines, and simultaneous treatment of ACD and SN-38 or SN-38 followed by ACD also resulted in synergistic effects to three cell lines. THP-adriamycin followed by SN-38 had synergistic effects to four cell lines. The present quantitative data analysis for synergism provides a basis for a rational design of clinical protocols for combination chemotherapy in patients with endometrial cancer of the uterus. [© 2000 Lippincott Williams & Wilkins.]

Key words: Anticancer drugs, cisplatin, combined effects, human endometrial cancer cell lines, irinotecan, peak plasma concentrations.

Introduction

In endometrial cancer, postoperative radiation therapy has been proven to reduce recurrence at primary sites.[1] However, distant failure outside of the treatment field is more common in patients whose tumors are high grade or deeply invasive,[2] so that adjuvant radiotherapy may not impact overall survival rate.[3] Tumors with deep myometrial invasion, high-grade, variant histology or extrauterine spread have a substantial rate of treatment failure. Adjuvant chemotherapy with cisplatin, doxorubicin and cyclophosphamide has been shown to confer a survival advantage in such a clinical setting.[4–6]

Recently, it was reported that paclitaxel (Taxol) was active in patients with advanced or recurrent endometrial cancer.[7,8] In addition, efficacy of irinotecan (CPT-11) or topotecan (topoisomerase I inhibitors) has been established in gynecologic cancer.[9] However, a rational role of CPT-11 or topotecan in treatment of endometrial cancer is still unknown.

Thus, we examined *in vitro* growth inhibitory effects of Taxol and CPT-11 on human endometrial cancer cell lines, and also its combination effects with other anticancer drugs.

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Materials and methods

Established cell lines

Ishikawa 3-H-12 cells were kindly provided by Dr Nishida.[10] HEC-1A, HEC-50B, HEC-59 and HEC-108 cells were kindly supplied by Dr Kuramoto.[11] Ishikawa cells were derived from well-differentiated adenocarcinoma of the endometrium. HEC-1A, HEC-50B and HEC-59 cells were established from moderately differentiated endometrial cancer. HEC-108 cells were derived from poorly differentiated adenocarcinoma of the endometrium. The cells were incubated in RPMI 1640 medium supplemented with 10% fetal calf serum, 2 mM glutamine, 100 U penicillin/ml and 100 μg streptomycin/ml (Gibco, Grand Island, NY) at 37°C in a 5% CO_2 incubator. The medium was changed every 3 days and the cells were passaged when they reached confluence.

Anticancer drugs

Cisplatin [*cis*-diamminedichloroplatinum(II)], 4′-dimethylepipodophyllotoxin-β-D-ethylidene glucoside (VP-16, etoposide) and paclitaxel (Taxol) were obtained from Bristol-Myers Squibb (Tokyo, Japan). SN-38, which is an active metabolite of irinotecan (CPT-11), was supplied by Daiichi Pharmaceutical (Tokyo, Japan). 5-Fluorouracil (5-FU), pirarubicin hydrochloride (THP), aclarubicin hydrochloride (ACR) and actinomycin-D (ACD) were obtained from Kyowa Hakko Kogyo (Tokyo, Japan), Meiji Seika (Tokyo, Japan), Yamanouchi Pharmaceutical (Tokyo, Japan) and Banyu Pharmaceutical (Tokyo, Japan), respectively. Since doxorubicine was not available in Japan, the efficacy of THP and ACR was examined in stead of doxorubicine. 4-Hydroperoxy ifosfamide (4-OHIFO), which is an active metabolite of ifosfamide (IFO), was provided from Shionogi Pharmaceutical (Osaka, Japan). Efficacy of each drug used in the present study was defined as sensitive when the IC_{50} dose was lower than 10% of plasma peak concentrations (PPC) as described previously.[12]

In vitro growth inhibition test

To examine concentrations of anticancer drugs required for 50% inhibition of cell growth *in vitro* (IC_{50}), 3000 cells/well in 100 μl of growth medium were seeded into 96-well flat-bottomed microtiter plates (Becton Dickinson, Mountain View, CA). After incubation for 24 h various concentrations of drugs diluted in 100 μl of the medium were added to each well and after an additional incubation for 72 h cytotoxicity of various anticancer drugs on each cell line was determined using a crystal violet staining method.[13] Briefly, an equal volume of 10% formalin phosphate-buffered saline (PBS) containing 0.2% crystal violet was added into each well and left at room temperature for 20 min. After washing twice with distilled water and drying at room temperature, optical density at 590 nm of stained cells in each well was measured by an automatic microtiter plate reader (Multiscan MCC/340, Titertek; Flow Laboratories, MacLean, VA). Average optical density of the control wells in the absence of anticancer drugs was regarded as 100% and the percentage cell growth in each well was calculated. The concentration that inhibited the growth of cells to the level of 50% of the control growth (IC_{50}) was obtained from graphical plots.

Median-effect analysis

Combined effects of paclitaxel and SN-38 with other anticancer drugs were analyzed by median-effect analysis as described previously.[14] Briefly, this method compares the effect of drug combinations to the effect of individual drugs across the entire dose–effect range. Paclitaxel or SN-38 was added simultaneously with other drugs and cells were incubated for 72 h. In sequential exposure experiments, after or before cells were treated with Taxol or SN-38 for 36 h, the other second drugs were added for 36 h. Growth inhibition data were then fitted to regression lines, and the concentration of each drug which produced a given level of growth inhibition [fractional effect (F_a)], alone or in combination, was determined. The combination index (CI) for a given F_a (typically 0.5) was calculated as follows, $CI=d_1/D_1+d_2/D_2$, where D_1 and D_2 are the doses of drugs 1 and 2, which by themselves produce a given F_a (i.e. IC_{50}); d_1 and d_2 are the doses which produce the same F_a in combination. CI=1 indicates zero interaction (additive cytotoxicity), $CI<1$ indicates synergy and $CI>1$ indicates anatagonism. Results were expressed as the mean $\pm$ SD. The statistical significance of the difference in the mean values was determined by Student's *t*-test.

Results

Effects of anticancer drugs on proliferation of endometrial cancer cell lines

Effects of paclitaxel, SN-38 and cisplatin on the proliferation of five endometrial cancer cell lines are shown in Table 1. IC_{50} values of paclitaxel to all cancer cell lines used in this study were lower than one-tenth the PPC, suggesting efficacy of paclitaxel to endome-

Table 1. Growth inhibitory effects (IC_{50})[a] of the key drugs in endometrial cancer cell lines

Cell lines	Taxol (nM)	SN-38 (nM)	Cisplatin (μM)
Ishikawa	5.2	7.9	2.8
HEC-1A	1.1	8.7	8.2
HEC-50B	21.5	66.6	3.3
HEC-59	6.5	5.1	3.9
HEC-108	3.9	20.2	7.5
PPC[b]	4680.0	124.9	5.0
Cut-off level[b]	468.0	12.5	0.5

[a]Concentration of drug required for 50% inhibition of cell growth *in vitro*. Each value is the average from three independent experiments.
[b]Peak plasma concentrations. The cut-off level is defined as one-tenth the PPC level as described in Materials and methods.

trial cancers. SN-38 had IC_{50} values lower than one-tenth the PPC to Ishikawa, HEC-1A and HEC-59 endometrial cancer cell lines. Cisplatin was not effective against any endometrial cancer cell lines examined in this study. The results of sensitivity tests to other anticancer drugs in endometrial cancer cell lines are shown in Table 2. Four of five endometrial cancer cell lines were sensitive to ACR and ACD, two of five cell lines were sensitive to VP-16 and THP, and only one cell line, Ishikawa, was sensitive to 5-FU. 4-OHIFO had no effect against any cell lines examined in this study. Among all endometrial cancer cell lines, the Ishikawa cell line, derived from well-differentiated (grade 1) adenocarcinoma, was the most sensitive to all anticancer drugs (except 4-OHIFO) used in this

Table 2. Growth inhibitory effects (IC_{50})[a] of various anticancer drugs in endometrial cancer cell lines

Cell lines	VP-16 (μM)	5-FU (μM)	THP (nM)	ACR (nM)	ACD (nM)	4-OHIF (μM)
Ishikawa	4.4	11.1	12.5	2.6	1.4	40.0
HEC-1A	2.1	36.7	10.0	1.3	0.8	40.0
HEC-50B	25.5	85.0	122.9	1.7	210.0	32.1
HEC-59	9.6	58.0	46.5	53.8	2.6	32.5
HEC-108	21.2	130.1	66.5	1.9	3.2	36.0
PPC[b]	51.0	117.6	159.3	235.8	477.9	90.1
Cut-off level[b]	5.1	11.8	15.9	23.6	47.8	9.0

[a]Concentrations of drug required for 50% inhibition of cell growth *in vitro*. Each value is the average from three independent experiments.
[b]Peak plasma concentrations. The cut-off level is defined as one-tenth the PPC level as described in Materials and methods.

Table 3. Combination indices (CI) of Taxol (Tx) with other drugs (D) in endometrial cancer cell lines

		SN-38	Cisplatin	VP-16	5-FU	THP	ACR	ACD	4-OHIFO
Ishikawa	Tx+D	1.37±0.20	1.56±0.07[b]	1.30±0.21	1.08±0.06	1.91±0.03[b]	1.71±0.21[b]	1.91±0.12[b]	2.16±0.46[b]
	Tx→D	0.92±0.28	0.98±0.26	1.37±0.05[b]	1.69±0.18[b]	1.73±0.14[b]	2.22±0.06[b]	2.07±0.14[b]	2.20±0.34[b]
	D→Tx	0.86±0.13	1.73±0.16[b]	0.75±0.18	1.81±0.18[b]	1.96±0.35[b]	1.92±0.11[b]	2.12±0.04[b]	2.72±0.66[b]
HEC-1A	Tx+D	0.95±0.15	1.31±0.17	1.05±0.05	1.21±0.18	1.83±0.31[b]	1.93±0.21[b]	1.95±0.13[b]	1.64±0.05[b]
	Tx→D	0.43±0.07[a]	0.75±0.10[a]	1.10±0.09	0.68±0.06[a]	0.64±0.17	1.55±0.36	2.34±0.25[b]	0.76±0.24
	D→Tx	1.03±0.13	2.25±0.22[b]	0.54±0.17[a]	1.17±0.13	0.81±0.08	2.15±0.22[b]	1.33±0.17	2.49±0.52[b]
HEC-50B	Tx+D	0.88±0.11	1.41±0.36	0.93±0.14	1.17±0.17	1.48±0.34	1.99±0.03[b]	1.00±0.01	1.19±0.10
	Tx→D	0.40±0.19[a]	0.77±0.25	0.53±0.08[a]	1.11±0.13	0.50±0.14[a]	1.53±0.16[b]	0.98±0.15	0.36±0.14[a]
	D→Tx	0.80±0.13	1.04±0.15	0.75±0.05[a]	1.83±0.09[b]	1.13±0.16	2.64±0.23[b]	1.28±0.14	1.24±0.16
HEC-59	Tx+D	1.17±0.37	1.50±0.25	1.36±0.27	0.68±0.18	1.63±0.02[b]	1.53±0.33	1.15±0.25	1.14±0.04[b]
	Tx→D	1.14±0.09	0.95±0.13	1.50±0.11[b]	0.77±0.14	1.29±0.12	1.85±0.07[b]	3.29±0.13[b]	0.79±0.29
	D→Tx	1.55±0.07[b]	1.19±0.39	0.94±0.07	0.88±0.11	0.91±0.12	1.15±0.05[b]	0.63±0.14[a]	1.26±0.13
HEC-108	Tx+D	1.25±0.05[b]	1.48±0.06[b]	1.85±0.09[b]	1.37±0.02[b]	1.39±0.41	1.92±0.12[b]	1.08±0.02[b]	1.41±0.18
	Tx→D	0.66±0.24	0.60±0.16[a]	1.12±0.38	0.77±0.21	0.71±0.13	0.94±0.16	2.89±0.05[b]	1.90±0.08[b]
	D→Tx	1.22±0.04[b]	1.26±0.14	1.60±0.08[b]	1.56±0.05[b]	0.87±0.24	1.43±0.10[b]	1.52±0.16[b]	0.73±0.10[a]

CI values were determined by median effect analysis at F_a (fractional effect) = 0.5. CI < 1, CI = 1 and CI > 1 indicate synergism (underlined), additive effect and antagonism, respectively. Values are means ± SD. (n = 3).
Tx + D: simultaneous exposure. Tx→D: sequential exposure of Taxol (Tx) followed by other drugs (D). D→Tx: sequential exposure of other drugs (D) followed by Taxol (Tx).
[a]Significantly synergistic ($p < 0.05$, compared to CI = 1).
[b]Significantly antagonistic ($p < 0.05$, compared to CI = 1).

Table 4. Combination indices (CI) of SN-38 (SN) with other drugs (D) in endometrial cancer cell lines

		Cisplatin	VP-16	5-FU	THP	ACR	ACD	4-OHIFO
Ishikawa	SN + D	0.71 ± 0.10[a]	1.36 ± 0.08[b]	1.16 ± 0.11	1.34 ± 0.05[b]	1.93 ± 0.11[b]	1.25 ± 0.13	1.46 ± 0.12[b]
	SN→D	1.40 ± 0.25	2.62 ± 0.14[b]	1.34 ± 0.10[b]	1.05 ± 0.13	2.50 ± 0.17[b]	0.32 ± 0.06[a]	0.68 ± 0.20
	D→SN	1.25 ± 0.13	2.97 ± 0.39[b]	1.68 ± 0.18[b]	0.97 ± 0.26	1.71 ± 0.13[b]	0.69 ± 0.15	1.43 ± 0.22
HEC-1A	SN + D	0.87 ± 0.04[a]	0.63 ± 0.22	0.91 ± 0.01[a]	1.13 ± 0.11	1.77 ± 0.28[b]	1.56 ± 0.33	1.19 ± 0.07[b]
	SN→D	0.88 ± 0.16	1.05 ± 0.09	1.04 ± 0.17	1.84 ± 0.17[b]	2.69 ± 0.12[b]	2.06 ± 0.14[b]	2.14 ± 0.21[b]
	D→SN	1.04 ± 0.07	0.28 ± 0.07[a]	1.00 ± 0.18	0.75 ± 0.10[a]	0.91 ± 0.28	0.60 ± 0.16[a]	1.79 ± 0.06[b]
HEC-50B	SN + D	0.66 ± 0.03[a]	0.91 ± 0.07	1.49 ± 0.07[b]	1.40 ± 0.05[b]	1.68 ± 0.14[b]	0.70 ± 0.12[a]	1.73 ± 0.03[b]
	SN→D	1.05 ± 0.12	1.85 ± 0.12[b]	1.22 ± 0.08[b]	0.90 ± 0.19	1.27 ± 0.29	0.92 ± 0.07	1.23 ± 0.19
	D→SN	1.02 ± 0.24	1.70 ± 0.23[b]	1.31 ± 0.20	1.38 ± 0.26	2.69 ± 0.33[b]	0.87 ± 0.12	1.99 ± 0.40[b]
HEC-59	SN + D	0.64 ± 0.02[a]	1.50 ± 0.26	0.76 ± 0.14	1.44 ± 0.21	0.65 ± 0.06[a]	0.82 ± 0.12	1.47 ± 0.30
	SN→D	0.93 ± 0.09	2.28 ± 0.16[b]	0.89 ± 0.13	1.27 ± 0.16	0.75 ± 0.05[a]	0.73 ± 0.17	1.27 ± 0.17
	D→SN	1.00 ± 0.12	0.98 ± 0.05	1.02 ± 0.14	0.62 ± 0.15[a]	0.72 ± 0.10[a]	0.35 ± 0.09[a]	1.53 ± 0.26
HEC-108	SN + D	0.75 ± 0.04[a]	1.67 ± 0.43	1.06 ± 0.09	1.23 ± 0.12	1.05 ± 0.22	0.93 ± 0.04	1.37 ± 0.16
	SN→D	1.05 ± 0.16	0.92 ± 0.04	1.08 ± 0.13	0.95 ± 0.20	1.50 ± 0.18[b]	1.35 ± 0.14[b]	2.08 ± 0.49
	D→SN	0.87 ± 0.09	1.45 ± 0.23	1.21 ± 0.14	0.74 ± 0.13	0.96 ± 0.13	0.83 ± 0.07[a]	2.57 ± 0.38[b]

CI values were determined by median effect analysis at F_a (fractional effect) = 0.5. CI < 1, CI = 1 and CI > 1 indicate synergism (underlined), additive effect and antagonism, respectively. Values are means ± SD. (n = 3).
SN + D: simultaneous exposure. SN→D: sequential exposure of SN38 (SN) followed by other drugs (D). D→SN: sequential exposure of other drugs (D) followed by SN38 (SN).
[a]Significantly synergistic ($p < 0.05$, compared to CI = 1).
[b]Significantly antagonistic ($p < 0.05$, compared to CI = 1).

study. HEC-1A, derived from moderately differentiated (grade 2) adenocarcinoma was the second most drug-sensitive cell line. Except for 5-FU, the sensitivity profile of HEC-1A was similar to that of the Ishikawa cell line.

Combined effects of paclitaxel and SN-38 with other anticancer drugs

Combination effects of paclitaxel with other drugs were determined in the five endometrial cancer cell lines by median-effect analysis (Table 3). Simultaneous combination of paclitaxel with cisplatin, THP, ACR or 4-OHIFO showed antagonistic effects (CI values at F_a=0.5) in all endometrial cancer cell lines. Simultaneous combination of paclitaxel with SN-38 seemed to be synergistic in HEC-1A and HEC-50B cell lines, while that of paclitaxel with VP-16 or 5-FU was synergistic only in HEC-50B or HEC-59 cell lines, respectively. Paclitaxel followed by cisplatin showed a synergistic effect in all endometrial cancer cell lines. When paclitaxel was followed by SN-38, these drugs showed a synergistic effect in all cell lines except HEC-59. In sequential exposure with paclitaxel and THP adriamycin, this drug combination had synerigistic effects in a schedule-independent manner. In sequential exposure with paclitaxel and the other drugs, no consistent effect on these cell lines was obtained. On the other hand, combination effects of SN-38 with other anticancer drugs in the endometrial cancer cell lines are shown in Table 4. Interestingly, when SN-38 was simultaneously combined with cisplatin, marked combination effects (CI values at F_a=0.5) were observed in all endometrial cancer cell lines used in this study, while simultaneous combination of SN-38 with THP or 4-OHIFO showed antagonistic effects in all endometrial cell lines examined. It is noteworthy that combination of SN-38 with ACD was most effective, especially when ACD was followed by SN-38. Similarly, THP-adriamycin was more effective when THP was followed by SN-38. Table 5 summarizes these results.

Table 5. Summary of drug combinations with the greatest synergy or antagonism

Synergy	Antagonism
SN-38 + cisplatin	Tx + or →ACD
ACD → SN-38	Tx + ACR or THP
Tx → cisplatin	Tx + 4-OHIFO
Tx → SN-38	Tx + cisplatin
VP-16 → Tx	4-OHIFO + or → SN-38
	SN-38 + or → ACR
	SN-38 → VP-16

Tx, paclitaxel.

Discussion

In the present study, we have demonstrated that the most active single agent to endometrial cancer cell lines was paclitaxel. Paclitaxel was active against all

endometrial cancer cell lines including not only grade 1 but also grade 2 and grade 3 cells (Table 1). These results coincide with the previous results of clinical trials.[15,16] They reported that paclitaxel was active against platinum-resistant endometrial adenocarcinoma with high histologic grade 2 or 3. Histologic grade was defined according to criteria of the International Federation of Gynecologists and Obstetricians (FIGO) as follows[17]: grade 1=5% or less of a non-squamous or non-morular solid growth pattern, grade 2=6–50% of a non-squamous or non-morular solid growth pattern and grade 3=more than 50% of a non-squamous or non-morular solid growth pattern. Therefore, it is possible that paclitaxel is promising not only in advanced endometrial cancer patients but also in high-risk endometrial cancer patients. In fact, efficacy of paclitaxel in patients with advanced or recurrent endometrial carcinoma had been reported.[7,8] In addition, SN-38, an active metabolite of CPT-11, was also sensitive to three endometrial cancer cell lines including histologic grade 1 and 2. Cisplatin has become established as a key anticancer agent for use in the treatment of gynecological malignancies. It has been used extensively to treat ovarian cancer and has achieved fairly good response rates. Cisplatin has also been found to be useful for endometrial cancer.[18,19] However, in the present *in vitro* study, the IC_{50} of cisplatin showed more than one-tenth the PPC levels to all endometrial cancer cell lines examined in this study. Although these results seem to conflict with the results of previous clinical trials, most such clinical studies have shown results obtained by a combination of cisplatin with other drugs but not the use of single-agent cisplatin.[5,20,21] As shown in Table 2, ACR and ACD seemed to be active against four endometrial cancer cell lines, while THP and VP-16 were active against two cell lines, and 5-FU was active against only one cell line. Although THP and ACR were used instead of doxorubicin in the present study, doxorubicin and epirubicin showed a 26% response rate when they were used as a single agent.[15] On the other hand, 4-OHIFO did not show activity against any endometrial cancer cell lines used in this study.

Based on the results of chemosensitivity by single agents, we selected Taxol and CPT-11 (SN-38) as new key drugs in combination with other drugs. When simultaneously combining paclitaxel with cisplatin, THP, ACR or 4-OHIFO, antagonistic effects rather than additive effects were observed as analyzed by median-effect analysis (Table 3). Synergistic effects were observed to two endometrial cancer cell lines by simultaneous combination with SN-38. Simultaneous combination with VP-16 or 5-FU resulted in synergistic effects to only one cell line. In the sequential combination of paclitaxel and cisplatin, when paclitaxel was followed by cisplatin synergistic effects were observed in all endometrial adenocarcinomas, as in ovarian adenocarcinoma. Judson *et al.*[22] reported that cisplatin blocks paclitaxel-induced apoptosis at a point downstream of Bcl-2 degradation and independent of microtubule stabilization, showing that cisplatin can inhibit the effectiveness of paclitaxel in cisplatin-resistant cells. All endometrial adenocarcinoma cell lines used in the present study had an IC_{50} of cisplatin more than one-tenth the PPC. Similarly, paclitaxel followed by SN-38 had synergistic effects in four cell lines, especially in two (HEC-50B and HEC-108) cell lines with higher IC_{50} of SN-38 than one-tenth the PPC, suggesting sensitization of SN-38 by paclitaxel. Even if THP-adriamycin was administered before or after paclitaxel administration, synergistic effects were obtained in three cell lines. Combination of paclitaxel with the other drugs was less effective. Since these results were obtained only by *in vitro* study, further study is necessary to confirm these results in the clinical situation. Recently it has been reported that cisplatin-, epirubicin- and paclitaxel-containing chemotherapy should be prospectively compared to standard combinations as initial treatment for advanced endometrial carcinoma.[23]

In combinations of SN-38 with other drugs, it is noteworthy that significant synergistic effects were obtained in all endometrial cancer cell lines when simultaneously combined with cisplatin and when ACD was followed by SN-38 (Table 4). Synergistic enhancement of cisplatin cytotoxicity by SN-38 in cisplatin-resistant HeLa cells has already been reported.[24] We confirmed these findings in all endometrial cancer cell lines including not only grade 1 but also grade 2 and grade 3. To our knowledge this is the first report of a synergistic effect of ACD followed by SN-38. We speculate that the mechanism is that the effect of ACD on DNA damage may be amplified by topoisomerse I inhibitor (SN-38). Thus, cisplatin or ACD combined with CPT-11 may be a new regimen with a great potency in endometrial carcinoma. Such a synergistic effect in the combination of CPT-11 with cisplatin or ACD may also warrant clinical trials.

In conclusion, when using drug combinations, schedule dependency or independency must be taken into consideration as shown in the present study.

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