SPANDIDOS PUBLICATIONS

Irinotecan HCl, an anticancer topoisomerase I inhibitor, frequently induces ovarian failure in premenopausal and perimenopausal women

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Received January 18, 2008; Accepted February 18, 2008

Abstract. The effects of irinotecan HCl (CPT-11) combination chemotherapies on the hypothalamus-pituitary-ovary endocrine system were examined clinically. The incidences of typical menopausal malaises and/or endocrinological findings were investigated in 32 gynecological cancer patients treated by CPT-11 combination chemotherapies. Patients who complained of menopausal malaises or had been treated by hormone replacement therapy before chemotherapy were excluded from the study. Menopausal malaise-like symptoms (MMLS) appeared in 6 of 32 patients (18.8%) during CPT-11 combination chemotherapy, and these symptoms were completely cured within a few days by administration of conjugated estrogen tablets (0.625 mg/day). All the MMLS cases were perimenopausal patients (47-57 years of age), and MMLS were not found in any of the postmenopausal patients who had exceeded 3 years since endocrinological menopause or patients who had recurrent cancer after pelvic radiotherapy. After exclusion of these 3-year-postmenopausal patients and postirradiation patients, 6 of 7 patients aged 45-59 years complained of MMLS during CPT-11 combination chemotherapy. The incidence of CPT-11-induced MMLS showed no relationships with the anticancer drugs combined with CPT-11, mean total CPT-11 dose, mean number of CPT-11 injections, mean individual CPT-11 dose, grade of CPT-11-specific diarrhea or anticancer effects of each CPT-11 combination chemotherapy. The perimenopausal cancer patients with CPT-11-induced MMLS showed decreased serum estradiol and increased serum FSH and LH levels accompanying the CPT-11 injections. A young patient with CPT-11-induced secondary amenorrhea showed decreased serum estradiol and increased serum FSH and LH levels accompanying the CPT-11 injections. None of the postmenopausal patients with high FSH and LH levels showed any significant differences in their serum FSH, LH, PRL and TSH levels during CPT-11 combination chemotherapy. No differences in the results of LHRH and TRH tests during chemotherapy were found for postmenopausal patients. Histopathological examinations of normal ovarian tissues surgically removed from 4 young cervical cancer patients treated with preoperative CPT-11 combination chemotherapies revealed no growing ovarian follicles in the ovarian tissues. CPT-11 injections can induce estrogen-rescued MMLS in cancer patients aged ~50 years at a very high rate and may induce secondary amenorrhea in young women. The endocrinological and histopathological studies revealed that CPT-11 causes ovarian follicular loss and ovarian failure within a short time without affecting hypothalamic and pituitary hormone secretion. These clinical results indicate that CPT-11 has strong ovarian toxicity and that repeated CPT-11 administrations may frequently induce ovarian follicular loss and premature ovarian failure, even in young women.

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Key words: CPT-11, irinotecan, ovarian failure, menopause, topoisomerase I inhibitor

Introduction

During anticancer chemotherapy, cancer patients complain of various adverse effects. Among the lethal adverse effects of anticancer drugs, the most frequent ones are myeloid suppressions, such as neutropenia, thrombocytopenia and anemia. These myeloid suppressions are caused by the death of immature bone marrow cells, which are highly proliferative and therefore sensitive to anticancer drugs. Loss of hair and digestive symptoms such as diarrhea, nausea, vomiting and abdominal pains are also frequent adverse effects of anticancer drugs. Some of these symptoms are caused by the death of intestinal mucoepithelial cells and hair stem cells, which also have high proliferative activities. Thus, normal cells with high proliferative activity can be the main targets of anticancer drugs, and the resulting cell death results in frequent common adverse effects for various chemotherapy regimens. Moreover, psychological symptoms such as sleeplessness, irritability and depression are often found among cancer patients as functional adverse effects of chemotherapy. Paclitaxel and cisplatin represent the most frequently used drugs for gynecological cancer patients, and are well known

to induce numbness and peripheral nerve paralysis. These psychological and neurological symptoms usually recover gradually after completion of the chemotherapy regimen.

Gonadal toxicity is a rare adverse side effect of anticancer drugs, but the damage caused may be permanent. Most reproductive cells in gonads tend to have such high proliferative activities that they are easily damaged by anticancer drugs. When young cancer patients are intensively treated with any anticancer chemotherapy associated with high gonadal toxicity, they may show decreased sex hormone productivity, reduced spermatogenetic activity or ovarian follicular loss. Finally, they may suffer from severe sterility after chemotherapy (1-3). Postpubertal male cancer patients are not usually affected by sterility, since cryopreservation of viable sperms from ejaculated semen before initiation of chemotherapy is sufficient to subsequently create embryos by *in vitro* fertilization (4). On the other hand, the numbers of ovarian follicles in young female cancer patients are limited and it is very difficult to cryopreserve many embryos or unfertilized oocytes before initiation of chemotherapy. Moreover, there are no reliable therapies for preventing or treating anticancer drug-induced ovarian failure (5-9).

Spontaneous ovarian failure with aging usually occurs as a spontaneous menopause ~50 years of age. It is well known that some spontaneous menopausal women aged ~50 years suffer from specific malaises, although most menopausal women do not complain of such malaises. When cancer patients are treated with a specific chemotherapy regimen associated with strong ovarian toxicity to induce rapid decreases in the serum estradiol level at a high rate, they often cannot adapt to the rapid decrease in serum estrogen and frequently complain of menopausal malaises. Estrogen-deprivation-specific menopausal symptoms, such as hot flashes and episodic sweating, can easily be cured by estrogen supplementation therapy, suggesting that any subacute onset of estrogen-relievable menopausal malaises during chemotherapy may be induced by the rapid anticancer drug-induced reduction in ovarian function.

Although sterility is considered to be a phenotype of the ovarian toxicity of anticancer drugs, recovery of the ovulatory cycle after anticancer chemotherapy cannot be clarified without endocrinological examinations. Therefore, endocrinological damage caused by anticancer drugs is usually unrecognized. Since severe ovarian failure can induce not only dysovulation but also amenorrhea, estrogen-rescued menopausal malaises associated with secondary amenorrhea must be a phenotype of the most severe ovarian toxicities of anticancer drugs. In fact, menopausal malaises as adverse effects of anticancer drugs are rarely reported and we have encountered few cancer patients with menopausal malaises caused by anticancer drugs (5-9). Although not all anticancer drugs exhibit severe gonadal toxicity, cyclophosphamide, chlorambucil, procarbazine, ifosfamide and busulfan are reported to have strong gonadal toxicities among anticancer drugs. On the other hand, 5-fluorouracil and drugs such as methotrexate, actinomycin-D and vinblastine, which are frequently used for treatment of trophoblastic diseases, are not associated with severe gonadal toxicity (10,11).

Irinotecan HCl (CPT-11), an anticancer prodrug, is converted into its main active metabolite, SN38, by carboxyl esterase in the body. SN38 is the most powerful inhibitor of topoisomerase I and shows strong antitumor effects by antagonizing DNA synthesis (12). CPT-11 has been used clinically in various cancer chemotherapies for uterine cancer (13-15), ovarian cancer (16,17), lung cancer (18), colorectal cancer (19,20), gastric cancer (21) and malignant lymphoma (22), and high response rates of these therapies have been reported. Although bone marrow suppression, such as neutropenia and thrombocytopenia, and digestive symptoms, such as nausea, vomiting, diarrhea and interstitial pneumonia, are well known as frequent severe adverse effects of CPT-11, no previous studies have investigated its gonadal toxicity. To the best of our knowledge, there is one report regarding CPT-11-induced gonadal dysfunction indicating that male rats injected with CPT-11 at 6 μg/g body weight exhibited reduced prostate and epididymis volumes (23), but CPT-11-induced ovarian toxicity was not examined in that study. Recently, CPT-11 combination chemotherapies have become major therapies for gynecological cancers. For example, CPT-11 combination chemotherapies have been shown to be highly effective for treating cervical cancers that have not been associated with positive chemotherapeutic effects for decades (13-15). Ovarian clear cell carcinomas are well known to be resistant to anticancer drugs such as taxan compounds and platinum drugs that are typically used in the main chemotherapy regimens for ovarian cancer patients. However, recent studies have reported high remission rates for ovarian clear cell carcinomas after treatment with CPT-11 combination chemotherapies (16,17).

We previously reported an ovarian cancer patient who complained of hot flashes and episodic sweating during CPT-11 combination chemotherapy (24). Subsequently, as we treated an increasing number of cancer patients, we found that CPT-11 combination chemotherapies induced menopausal malaise-like symptoms (MMLS), such as hot flashes, episodic sweating and irritability, more frequently than other chemotherapy regimens. Therefore, in the present study, we retrospectively and prospectively analyzed all the patients treated by CPT-11 combination chemotherapies under the care of one gynecologic oncologist and endocrinologist. Comparisons of the incidence of MMLS and clinical backgrounds of the patients were carried out. In addition, after obtaining informed consent, endocrinological examinations were performed during CPT-11 combination chemotherapy to clarify the mechanisms of CPT-11-induced ovarian failure.

Patients and methods

Patients. All the gynecologic cancer patients treated by CPT-11 combination chemotherapy under the care of one of the authors (Tetsuji Tanaka), a gynecologic oncologist and endocrinologist, at our university hospital from 2001 to 2006 were examined in this study. Patients who had been treated with hormone replacement therapy or had complained of menopausal malaises before initiation of CPT-11 combination chemotherapy were excluded from the study. The clinical data for the patients investigated in this study are summarized in Table I. The incidence of MMLS during chemotherapy was compared with clinical findings such as the age at chemotherapy, total dose of CTP-11 administered (or total dose

Table I. Characteristics of the cancer patients treated by CPT-11 combination chemotherapies in this study and their clinical results.

Case	Age	Primary cancer	Clinical stage or recurrence	Drug combined with CPT-11[a]	Total CPT-11 dose (mg)	No. of injections	Mean CPT-11 dose (mg)	Anti-tumor effect[c]	MMLS, hot flashes (grade[b])	Diarrhea (grade[b])	Histology of the primary cancer[d]	Pelvic radiotherapy before CPT-11 chemotherapy	Ovariectomy before CPT-11 chemotherapy	Chemotherapy before CPT-chemotherapy[a]
1	28	Ovary	IV	MMC	280	2	140	SD	0	0	CCC	No	Yes	CDDP
2	32	Cervix	Metastatic rec.	MMC	780	3	260	PR	0	1	SCC	Yes	No	DOC+NPL
3	35	Cervix	Ib	5-FU	420	2	210	PD	0	0	MA	Yes	Yes	None
4	40	Cervix	Metastatic rec.	CDDP, CBDCA	580	4	145	SD	0	1	SCC	Yes	No	BOMP, CAP, PTX+CBDCA
5	42	Cervix	IIIb	CDDP	720	9	80	PR	0	2	SCC	No	No	None
6	43	Cervix	Metastatic rec.	MMC	830	5	166	SD	0	0	CCC	No	Yes	None
7	44	Cervix	IIIb	MMC, CDDP	680	6	113	CR	0	1	SCC	Yes	No	None
8	47	Cervix	IVb	MMC	840	3	280	SD	0	0	SCC	No	Yes	None
9	**47**	**Cervix**	**IIIb**	**NPL**	**430**	**4**	**107.5**	**PR**	**3**	**3**	**SCC**	**No**	**No**	**None**
10	**49**	**Ovary**	**IV**	**MMC**	**1020**	**6**	**170**	**CR**	**3**	**3**	**CCC**	**No**	**No**	**None**
11	**51**	**Ovary**	**IIIc**	**CDDP**	**720**	**9**	**80**	**PR**	**3**	**0**	**SA**	**No**	**No**	**None**
12	**51**	**Ovary**	**IV**	**THP, DOC**	**400**	**4**	**100**	**SD**	**2**	**0**	**UC**	**No**	**No**	**PTX+CDDP, PTX+CBDCA**
13	**52**	**Cervix**	**IIIb**	**MMC**	**800**	**5**	**160**	**PR**	**2**	**0**	**SCC**	**No**	**No**	**None**
14	53	Cervix	IIIb	MMC	420	3	140	PR	0	3	SCC	No	No	None
15	53	Ovary	IIIc	CDDP	2430	27	90	NE	0	2	MA	No	Yes	None
16	53	Ovary	Metastatic rec.	ADM, THP	720	18	40	CR	0	0	CCC	No	Yes	CPA+CBDCA, DOC+CBDCA
17	54	Cervix	Metastatic rec.	MMC	860	7	123	PR	0	3	SCC	Yes	No	NPL+IFO+PEM
18	55	Cervix	Metastatic rec.	MMC	800	5	160	PR	0	0	SCC	Yes	No	None
19	55	Ovary	Metastatic rec.	CDDP	360	2	180	PR	0	3	EA	No	Yes	CAP, PTX+CBDCA, PTX+CDDP, DOC+CBDCA
20	56	Oviduct	Metastatic rec.	ADM, THP	2295	35	65.6	CR	0	0	SA	No	Yes	PTX+CBDCA
21	**57**	**Ovary**	**IIc**	**CDDP**	**270**	**3**	**90**	**NE**	**1**	**0**	**MA**	**No**	**No**	**None**
22	59	Cervix	Metastatic rec.	MMC	920	7	131	PR	0	3	SCC	Yes	Yes	5-FU
23	60	Cervix	Ib	NPL	384	4	96	NE	0	1	SmCC	No	Yes	None
24	60	Ovary	IV	CBDCA	540	6	90	PR	0	0	Cs	No	Yes	None
25	60	Ovary	Metastatic rec.	CDDP	1080	12	90	PR	0	0	TCC	No	Yes	None
26	61	Cervix	IIIb	MMC	1100	6	183	CR	0	1	SCC	Yes	No	None
27	62	Ovary	IV	MMC	760	3	253	PR	0	0	CCC	No	Yes	PTX+CBDCA
28	62	Cervix	Pelvic rec.	MMC	1260	9	140	CR	0	0	CCC	Yes	Yes	None
29	63	Cervix	IVa	MMC, CDDP	1120	4	280	CR	0	3	SCC	Yes	No	None
30	65	Corpus	Metastatic rec.	MMC	360	3	120	SD	0	0	Cs	No	Yes	CDDP, VP16, DOC, CBDCA, CPA
31	69	Cervix	IIIb	MMC	800	5	160	PR	0	1	SCC	No	No	None
32	69	Corpus	Metastatic rec.	MMC	420	3	140	SD	0	0	EA	Yes	Yes	THP+VP16+CDDP

[a]Anticancer drugs: CDDP, cisplatin; CBDCA, carboplatin; NPL, nedaplatin; MMC, mitomycin C; THP, terarubicin; ADM, adriamycin; 5-FU, 5-fluorouracil; CPA, cyclophosphamide; IFO, ifomide; PTX, paclitaxel; DOC, docetaxel; VP16, etoposide; PEM, peplomycin; CAP, CPA+ADM+CDDP; CP, CPA+CDDP; BOMP, bleomycin+oncovin+MMC+CDDP. [b]Grades of hot flashes and diarrhea were evaluated according to the Common Terminology Criteria for Adverse Events v3.0 (CTCAE v3.0) (2003). Menopausal malaise-like symptoms (MMLS) were graded by the severity of the hot flashes. [c]Antitumor effects of the chemotherapy: CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; NE, not evaluated; rec., recurrence. [d]CCC, clear cell carcinoma; SCC, squamous cell carcinoma; MA, mucinous adenocarcinoma; SA, serous adenocarcinoma; UC, undifferentiated carcinoma; EA, endometrioid adenocarcinoma; SmCC, small cell carcinoma; Cs, carcinosarcoma. TCC, transitional cell carcinoma.

of CPT-11 administered before onset of MMLS during chemotherapy), total number of CPT-11 injections (or total number of CPT-11 injections before onset of MMLS during chemotherapy), mean CPT-11 dose administered (or mean CPT-11 dose administered before onset of MMLS during chemotherapy), anticancer effects of CPT-11 combination chemotherapy, anticancer drugs combined with CPT-11 and grading of CPT-11-specific diarrhea.

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

Endocrinological study. During the CPT-11 combination chemotherapy, endocrinological examinations were performed for patients who provided informed consent. The thyroid-stimulating hormone (TSH) assay kit used in our hospital was changed during the present study, and therefore different normal values are described in each figure. Both LH-releasing hormone (LHRH) and TSH-releasing hormone (TRH) tests were repeated on the postmenopausal patients after every CPT-11 injection after receiving informed consent. For the LHRH test, 0.1 mg of LHRH was intravenously injected into the patient, and the serum LH and FSH levels were assayed at 0, 30 and 60 min after the injection. For the TRH test, 0.5 mg of TRH was intravenously injected into the patient, and the serum TSH and prolactin (PRL) levels were assayed at 0, 30 and 60 min after the injection.

Histopathological study. Normal ovarian tissues surgically removed from 4 premenopausal cervical cancer patients during the study period were examined histopathologically. These 4 patients each received 4-5 CPT-11 injections and all had a radical hysterectomy with bilateral ovariectomy about 1 month after the last CPT-11 injection. One of these 4 patients is Case 9 in Table I, while the other 3 patients were treated by CPT-11 combination chemotherapies by three other gynecologists in our hospital, rather than by the authors.

Results

CPT-11 combination chemotherapy rapidly induces menopausal malaises in perimenopausal patients. The clinical data for all 32 patients investigated in this study are summarized in Table I. MMLS appeared in 6 of these 32 patients, and the symptoms of all 6 patients were completely relieved within a few days by administration of conjugated estrogen tablets (0.625 mg/day). All the patients with MMLS during CPT-11 combination chemotherapy were perimenopausal women (47-57 years of age). No MMLS occurred in patients aged >60 years or <45 years. Regarding the patients aged <60 years, estrogen-relievable MMLS did not appear in 1 postmenopausal patient at 3 years after spontaneous menopause (Case 14), 5 patients at least 2 years after ovariectomy (Cases 15, 16, 19, 20, and 22; 5/5, 100%) or 8 recurrent cancer patients after pelvic radiotherapy (Cases 2-5, 7, 17, 18 and 22; 8/8, 100%). Two young patients (Cases 1 and 5), who did not have ovariectomy or radiotherapy, did not complain of MMLS during chemotherapy. Although Case 8 was treated three times with CPT-11 and mitomycin (MMC) within 2 months after radical hysterectomy and bilateral ovariectomy for advanced cervical cancer, she did not complain of MMLS. Interestingly, she did not suffer from any myelosuppression, even though she was administered high doses of CPT-11. Since all MMLS were easily relieved by oral administration of conjugated estrogen tablets alone, the CPT-11-induced MMLS were considered to be estrogen-deprivation MMLS. We were unable to examine whether Case 21, whose uterus had been removed 9 years previously due to uterine leiomyoma, was endocrinologically premenopausal or postmenopausal before initiation of CPT-11 injections. However, she complained of MMLS after administration of small doses of CPT-11 and her MMLS were easily cured by estrogen therapy. Excluding the patients who were previously treated by pelvic radiotherapy or beyond 3 years after menopause, MMLS during CPT-11 combination chemotherapy occurred in 6 of 7 patients (86%) aged 45-59 years.

The antitumor effects of the CPT-11 combination therapies were very high. Among 29 patients with measurable tumors, CPT-11 combination chemotherapy showed CR in 24.1% (7/29) and PR in 48.3% (14/29) of patients. The overall response rate (CR+PR) was 72.4% (21/29). No relationship was found between the incidence of CPT-11-induced MMLS and chemotherapeutic anticancer efficiency. There was also no relationship between the incidence of CPT-11-induced MMLS and grading of diarrhea, a CPT-11-specific adverse effect.

As shown in Table II, the mean total CPT-11 dose administered before MMLS appeared in the MMLS-susceptible patients was 606.7±260.9 mg (range 270-1020 mg). The mean total CPT-11 dose administered to the patients without MMLS aged <50 years was 641.3±188.8 mg (range 280-840 mg), while that administered to the patients without MMLS aged >50 years was 923.8±579.3 mg (range 360-2430 mg). No relationships were found between the incidence of CPT-11-induced MMLS and the total CPT-11 doses administered.

The mean number of CPT-11 injections administered before MMLS appeared in the MMLS-susceptible patients was 5.2±2.0. The mean number of CPT-11 injections administered to the patients without MMLS aged <50 years was 4.3±2.2, while the number administered to patients without MMLS aged >50 years was 8.8±8.8. No relationships were found between the incidence of CPT-11-induced MMLS and the numbers of CPT-11 injections administered (Table II).

The mean CPT-11 dose administered before MMLS appeared in the MMLS-susceptible patients was 117.9±34.5 mg (range 80-170 mg). The mean CPT-11 dose administered to patients without MMLS aged <50 years was 174.3±65.7 mg (range, 80-280 mg), while that administered to patients without MMLS aged >50 years was 137.9±58.8 mg (range 40-280 mg). No relationships were found between the incidence of CPT-11-induced MMLS and the mean CPT-11 doses (Table II).

There were also no relationships between the incidence of CPT-11-induced MMLS and the anticancer drugs used in combination with CPT-11 (Table I).

Comparisons of the patients with and without CPT-11-induced MMLS.

Patients and MMLS	n	Case nos. in Table I	Patients beyond 2 years after endocrinological menopause or postirradiation patients	Mean age	Mean total dose of CPT-11 (mg)	Mean no. of CPT-11 injections (courses)	Mean individual dose of CPT-11 (mg/injection)	Overall response rate in the evaluated patients (CR+PR)[a]
Patients with CPT-11-induced MMLS	6	Cases 9-13 Case 21	0/5 (0%) Not classified: Case 8	51.2±3.1 (47-57)	606.7±260.9 (270-1020)	5.2±2.0 (3-9)	117.9±34.5 (80-170)	4/5 (80%)
Patients aged <50 years without CPT-11-induced MMLS	8	Cases 1-8	5/8 (62.5%) Cases 2-4, 6, 7	38.9±6.1 (28-47)	641.3±188.8 (280-840)	4.3±2.2 (2-9)	174.3±65.7 (80-280)	3/8 (37.5%)
Patients aged >50 years without CPT-11-induced MMLS	18	Cases 14-20 Cases 22-32	18/18 (100%)	59.4±5.0) (53-69	923.8±579.3 (360-2430)	8.8±8.8 (2-27)	137.9±58.8 (40-280)	14/16 (87.5%)

[a]CR, complete response; PR, partial response.

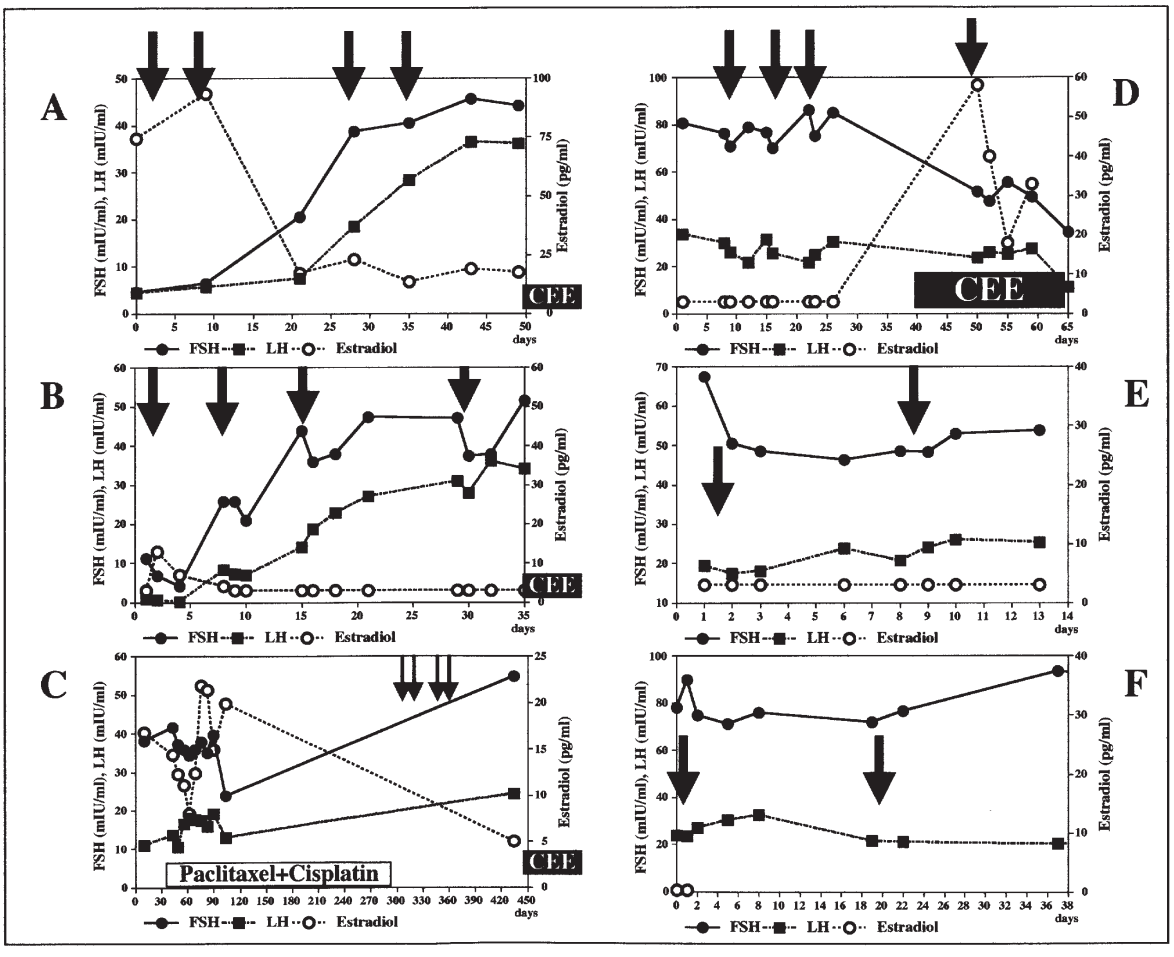

Figure 1. Endocrinological changes in cancer patients during CPT-11 combination chemotherapy. Open circles with dotted lines, serum estradiol levels; closed circles with solid lines, serum FSH levels; closed squares with dotted lines, serum LH levels; thick arrows, CPT-11 injections; CEE, treatment with conjugated estrogen tablets (0.625 mg/day). A, Case 9; B, Case 11; C, Case 12; D, Case 13; E, Case 25; F, Case 31.

Endocrinological studies of cancer patients during CPT-11 combination chemotherapy. Endocrinological examinations during CPT-11 combination chemotherapy were performed on cancer patients who provided informed consent. Premenopausal patients with estradiol secretion showed estradiol decreases to undetectable levels and increases in serum FSH and LH levels after injection of CPT-11 (Fig. 1A and B). Case 9 (Fig. 1A) and Case11 (Fig. 1B) showed CPT-11-induced

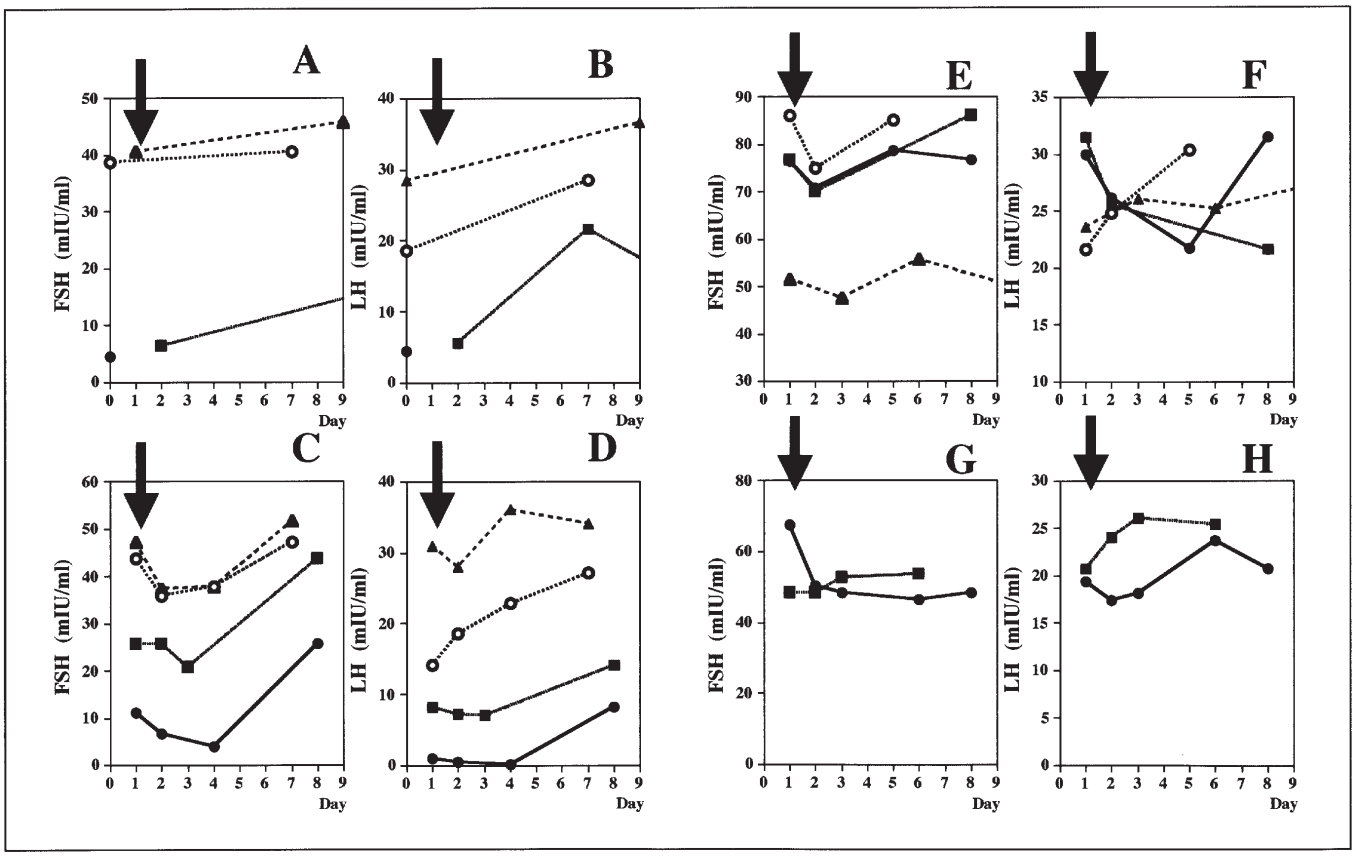

Figure 2. Changes in serum FSH and LH levels after every CPT-11 injection. A, C, E and G, serum FSH levels after CPT-11 injections; B, D, F and H, serum LH levels after CPT-11 injections. A and B, Case 9; C and D, Case 11; E and F, Case 13; G and H, Case 25. Closed circles with solid lines, hormonal changes after the first CPT-11 injection; closed squares with solid lines, hormonal changes after the second CPT-11 injection; open circles with dotted lines, hormonal changes after the third CPT-11 injection; open triangles with dotted lines, hormonal changes after the fourth CPT-11 injection.

MMLS, but their symptoms were easily relieved within a few days by oral administration of conjugated estrogen tablets.

Postmenopausal patients with detectable estradiol levels showed stable serum hormone levels with low estradiol, high FSH and high LH levels during CPT-11 combination chemotherapy (Fig. 1D-F), suggesting that CPT-11 did not directly affect FSH and LH secretion from the pituitary gland. However, Case 13 with undetectable serum estradiol complained of CPT-11-induced MMLS (Fig. 1D), although her symptoms were also easily cured by estrogen therapy. The findings for Case 13 suggest the following three possibilities: i) CPT-11 suppresses a very small amount of estrogen production in postmenopausal patients; ii) CPT-11 inhibits estrogen functions; and iii) CPT-11 directly causes MMLS. Since MMLS have never been induced in elderly patients by CPT-11 injections, it is highly unlikely that CPT-11 directly causes MMLS.

Case 12, a postmenopausal stage IV inoperable ovarian cancer patient with estradiol production (serum estradiol, 18-53 pg/ml) (Fig. 1C), was initially treated with 23 injections of combination chemotherapy with paclitaxel (PTX) and a platinum drug [carboplatin (CBDCA) or cisplatin (CDDP)]. Neither PTX plus CBDCA nor PTX plus CDDP induced MMLS in this patient or caused any decreases in the serum estradiol levels. Since she suffered from acquired anaphylactoid shocks from administration of PTX, CBDCA and CDDP, CPT-11 and pirarubicin (THP) were administered four times, and this was sufficient to induce MMLS with decreased serum estradiol and increased serum FSH levels. Conjugated estrogen therapy easily relieved her MMLS. This case suggests the possibility that CPT-11 suppresses a small amount of estrogen production from ovaries in postmenopausal women, but cannot exclude the possibility that CPT-11 inhibits estrogen function.

The changes in the serum FSH and LH levels after every CPT-11 injection were examined. As illustrated by Case 9 (Fig. 2A and B) and Case 11 (Fig. 2C and D), premenopausal patients with CPT-11-inuced MMLS showed increased serum FSH and LH levels after each CPT-11 injection, until their serum FSH and LH levels finally reached stable post-menopausal levels. However, the daily hormonal changes in the FSH and LH levels for each patient were similar for each CPT-11 injection (Fig. 2A-D). On the other hand, post-menopausal patients with undetectable serum estradiol, high FSH and high LH levels showed no daily changes in their serum FSH and LH levels after each CPT-11 injection (Fig. 2E-H).

The results shown in Figs. 1 and 2 suggest that CPT-11 suppresses serum estradiol within a short time and induces amenorrhea and MMLS in premenopausal women aged

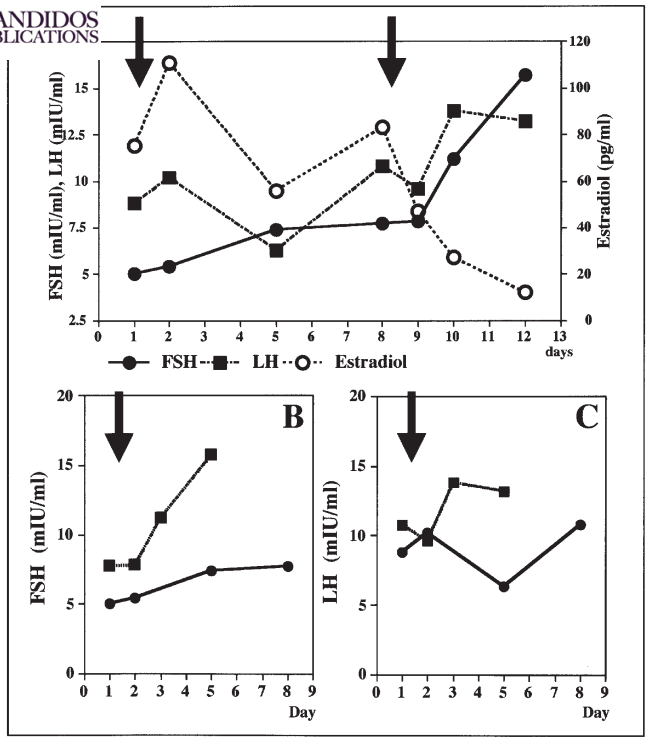

Figure 3. Endocrinological changes in a 28-year-old ovarian cancer patient (Case 1) during CPT-11 combination chemotherapy. A, Open circles with dotted lines, serum estradiol levels; closed circles with solid lines, serum FSH levels; closed squares with dotted lines, serum LH levels; thick arrows, CPT-11 injections. B, Serum FSH levels after CPT-11 injections. Closed circles with solid lines, hormonal changes after the first CPT-11 injection; closed squares with solid lines, hormonal changes after the second CPT-11 injection. C, Serum LH levels after CPT-11 injections. Closed circles with solid lines, hormonal changes after the first CPT-11 injection; closed squares with solid lines, hormonal changes after the second CPT-11 injection.

~50 years, and also that CPT-11 suppresses estradiol production and induces MMLS in postmenopausal women with a small amount of estrogen production. These results suggest the hypothesis that, in young women with high estrogen-producing ability, CPT-11 may suppress estrogen production without causing MMLS. In order to verify this hypothesis, we examined the endocrinological changes in young patients treated with CPT-11 after receiving informed consent. Fig. 3 shows the serum hormone changes in a 28-year-old ovarian cancer patient who received two injections of CPT-11 and MMC (Case 1). She developed grade 4 severe neutropenia after this chemotherapy and additional CPT-11 injections were stopped. Although she did not complain of MMLS during the CPT-11 combination chemotherapy, she had secondary amenorrhea accompanied by reduced serum estradiol and increased serum FSH and LH levels. Menstruation in this patient recovered at 2 months after the last CPT-11 injection when she was treated with PTX and CBDCA. The findings for this young patient demonstrate that CPT-11 induced the same endocrinological conditions as those shown in Fig. 1A and B, even though she did not have CPT-11-induced MMLS.

As shown in Figs. 1 and 3, CPT-11 decreased the serum estradiol levels and increased the serum levels of the pituitary hormones FSH and LH. Therefore, the effects of CPT-11 on other pituitary hormones, such as TSH and PRL, were investigated in patients after informed consent was obtained. In Case 9, CPT-11 decreased serum estradiol and increased serum FSH and LH levels, but did not induce any remarkable changes in the serum TSH and PRL levels during CPT-11 combination chemotherapy (Fig. 4A). In Case 31, serum TSH varied during CPT-11 chemotherapy within the normal

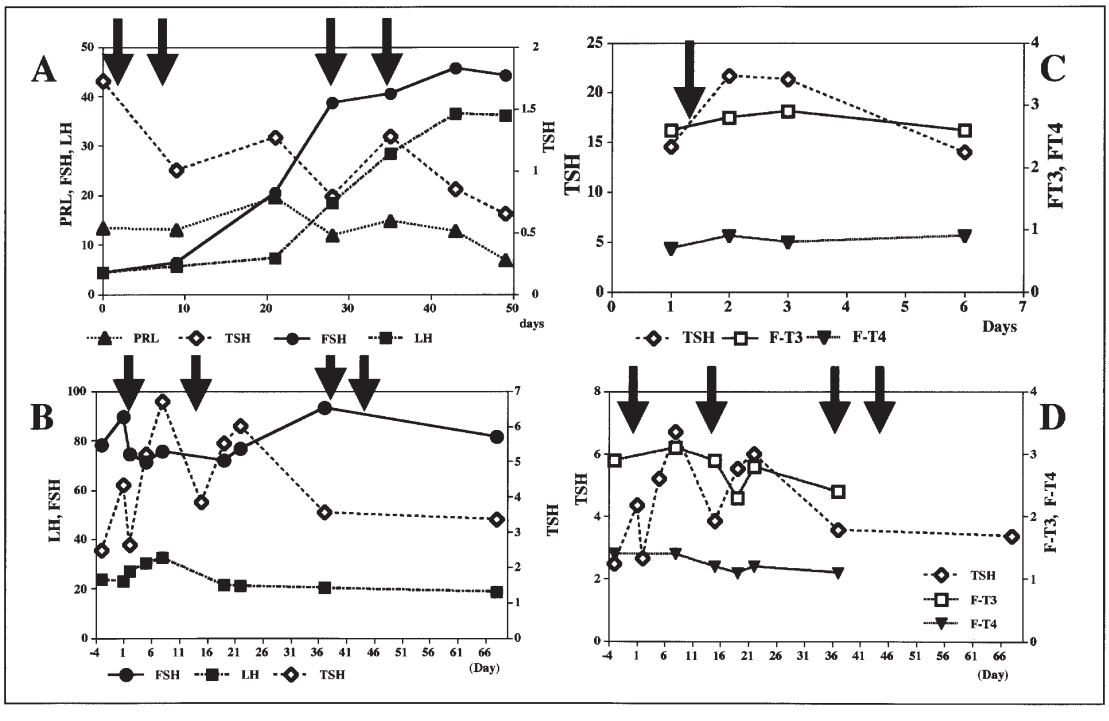

Figure 4. Changes in serum TSH and PRL levels during CPT-11 combination chemotherapy. Closed circles with solid lines, serum FSH levels; closed squares with dotted lines, serum LH levels; closed triangles with dotted lines, serum PRL levels (normal PRL <15 ng/ml); open diamonds with dotted lines, TSH levels (normal TSH, 0.5-4 mU/l in A; 0.34-3.5 ng/ml in B-D); closed triangles with solid lines, serum-free T4 (F-T4) levels (normal F-T4, 0.6-1.8 ng/dl); open squares with solid lines, serum-free T3 (F-T3) levels (normal F-T3, 3-5.5 pg/ml); thick arrows, CPT-11 injections. A, Case 9; B, Case 31; C, Case 25; D, Case 31.

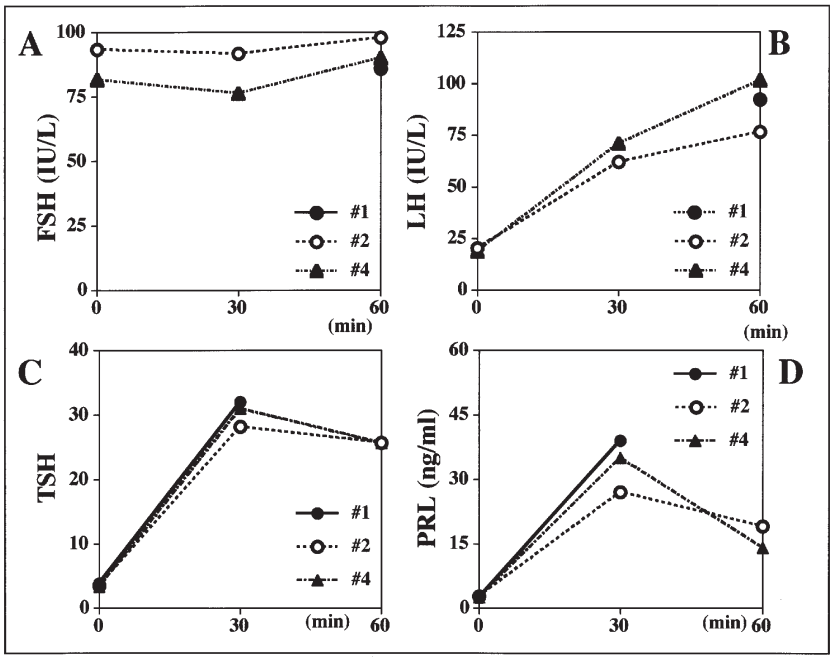

Figure 5. Results of LHRH and TRH tests for a postmenopausal cancer patient during CPT-11 combination chemotherapy. LHRH and TRH tests were performed for Case 31 after every CPT-11 injection. Closed circles with solid lines, hormonal changes after the first CPT-11 injection; open circles with dotted lines, hormonal changes after the second CPT-11 injection; open triangles with dotted lines, hormonal changes after the fourth CPT-11 injection. A, FSH changes evaluated by LHRH tests; B, LH changes evaluated by LHRH tests; C, TSH changes evaluated by TRH tests; D, PRL changes evaluated by TRH tests. Normal TSH in this study: 0.34-3.5 ng/ml.

Table III. Cervical cancer patients whose normal ovarian tissues were examined histopathologically after preoperative CPT-11 combination chemotherapies.

Case	Age	Primary cancer	Histology of the primary cancer	Clinical stage	Combination chemotherapy	Total pre-operative dose of CPT-11 (mg)	No. of CPT-11 injections	Days from the last preoperative CPT-11 injection to ovariectomy	Effects of CPT-11 on the cervical cancer (determined by MRI)
9	47	Cervical cancer	SCC	IIIb	CPT-11 + nedaplatin	430	4	28	CR
33	33	Cervical cancer	SCC	IIIb	CPT-11 + mitomycin	730	5	28	PR
34	44	Cervical cancer	SCC	IIb	CPT-11 + mitomycin	576	4	32	PR
35	31	Cervical cancer	SCC	Ib2	CPT-11 + nedaplatin	360	4	24	SD

CR, complete response; PR, partial response; SD, stable disease. SCC, squamous cell carcinoma.

range and no remarkable changes in the serum PRL levels were found (Fig. 4B and D). In Case 25, no remarkable changes in the serum-free T3, serum-free T4 and serum TSH levels were found (Fig. 4C).

In order to clarify that therapeutic doses of injected CPT-11 do not suppress hormone secretion from the pituitary gland, the pituitary hormone-secreting abilities were examined by LHRH and TRH tests. As shown in Fig. 5, the FSH-secreting and LH-secreting activities after stimulation with LHRH were not affected by CPT-11 injections (Fig. 5A and B). Moreover, the TSH-secreting and PRL-secreting activities after stimulation with TRH were not affected by CPT-11 injections (Fig. 5C and D).

Histopathological studies of normal ovaries resected from cervical cancer patients 1 month after the last preoperative CPT-11 injection. To investigate the morphological changes in ovaries from patients treated with CPT-11, histopathological studies were performed on normal ovaries resected from 4 premenopausal cervical cancer patients after preoperative CPT-11 combination chemotherapy under the care of 4 different gynecologists. The ovaries were removed ~1 month after the last CPT-11 injection. The clinical data for the 4 patients are summarized in Table III. Although these 4 young patients must have had regular menstrual cycles and many developing follicles in their ovaries before chemotherapy, considering their ages, there were no growing follicles in the

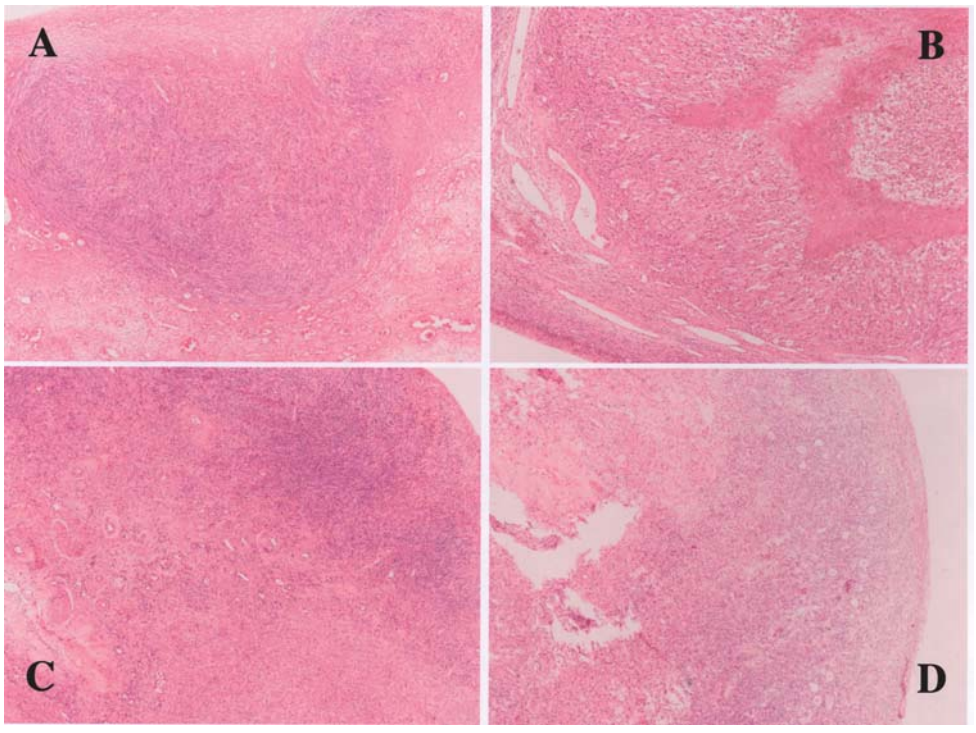

Figure 6. Histopathological findings for normal ovaries from 4 young cervical cancer patients after preoperative CPT-11 combination chemotherapy. The 4 patients were treated with preoperative CPT-11 combination chemotherapies by 4 different gynecologists. All the ovaries show almost total loss of growing follicles in the ovarian tissues. The clinical data for these 4 patients are summarized in Table III. A, Case 9; B, Case 33; C, Case 34; D, Case 35.

4 surgically removed ovaries (Fig. 6), indicating CPT-11-induced ovarian follicular loss.

Discussion

Cytotoxic anticancer drugs have additional effects on tissues with rapid cellular turnover rates. Although any damage caused by anticancer drugs to the bone marrow, gut and hair growth is reversible, such damage is progressive, permanent and irreversible in the ovaries, where the number of germ cells is limited and cannot be regenerated. Ovarian failure manifests itself as hypergonadotropic hypogonadism, resulting in amenorrhea and usually irreversible infertility. Once all the follicles with their oocytes have been damaged, there is no chance that contraception will ever occur with the patient's own oocytes. Cytotoxic anticancer chemotherapy frequently needs to be used in young cancer patients, in whom preservation of gonadal function and fertility is important. However, no standard treatments for preventing chemotherapy-related premature ovarian failure are currently available.

The present endocrinological study is the first report to show that CPT-11, an anticancer topoisomerase 1 inhibitor, induces ovarian failure in premenopausal women within a short time. The perimenopausal cancer patients examined complained of MMLS at a very high rate, regardless of the type of CPT-11 combination chemotherapy administered. To the best of our knowledge, there are no previous reports on CPT-11-induced ovarian failure and MMLS. No relationships were found between the incidence of MMLS and the various anticancer drugs combined with CPT-11, or between the incidence of MMLS and CPT-11-specific diarrhea. The incidence of CPT-11-induced MMLS was not related to the mean total CPT-11 dose, mean number of CPT-11 injections or mean individual CPT-11 dose. CPT-induced MMLS occurred in the perimenopausal patients at a very high rate (6/7, 86%). All the patients with MMLS were treated with 0.625 mg/day of conjugated estrogen and their symptoms were completely cured within a few days, indicating that estrogen-deprivation is the main cause of the CPT-11-induced MMLS. Moreover, postmenopausal cancer patients who had spontaneous menopause within 2 years previously complained of CPT-11-induced MMLS. These findings indicate the possibility that CPT-11 inhibits a small amount of estrogen production in the postmenopausal period as well as the possibility that CPT-11 inhibits estrogen functions. The fact that no CPT-11-induced MMLS appeared in patients aged >60 years may coincide with the fact that typical spontaneous menopausal malaises appear in perimenopausal patients and not in elderly patients. Since elderly patients have already acquired adaptation to estrogen-deprivation, they may not suffer from CPT-11-induced MMLS. These results suggest that CPT-11 does not induce MMLS directly and that CPT-11 only induces MMLS in perimenopausal patients aged >50 years due to the rapid endocrinological changes induced by CPT-11. The symptoms of all the patients with CPT-11-induced MMLS were completely cured by estrogen supplementation, indicating that CPT-11-induced MMLS were not directly caused by CPT-11.

After providing informed consent, some patients underwent endocrinological examinations during CPT-11 combination chemotherapy. The premenopausal patients aged ~50 years before administration of CPT-11 injections complained of

MMLS within a short time, accompanied by decreased serum estradiol, elevated serum FSH and elevated LH levels. In postmenopausal patients with high FSH and LH levels before CPT-11 administration, CPT-11 injections did not change the serum FSH and LH levels, and their responses in LHRH tests were not affected after repeated CPT-11 injections. These findings indicate that CPT-11 does not directly affect FSH and LH secretion from the pituitary gland. During CPT-11 injection therapy, the serum TRH and PRL levels remained unchanged. The responses in TRH tests also remained unchanged after repeated CPT-11 injections, although only a few patients were examined. These findings suggest that CPT-11 injections do not affect TSH and PRL secretion from the pituitary gland, similar to the case for FSH and LH secretion. The facts that CPT-11 did not affect the secretion of pituitary hormones such as FSH, LH, TSH and PRL in elderly cancer patients indicate that therapeutic doses of CPT-11 do not affect the secretion of hypothalamic hormones such as LHRH and TRH that regulate pituitary hormone production and secretion. Taking the results of these endocrinological examinations together, CPT-11 is considered to directly inhibit estradiol secretion from ovaries without any direct effects on the production and secretion of hypothalamic and pituitary hormones. Decreased estrogen secretion from ovaries is thought to induce secondary elevation of serum FSH and LH in a negative feedback endocrine regulatory mechanism. In fact, only two CPT-11 injections administered to a 28-year-old cancer patient were sufficient to cause transient secondary amenorrhea complicated with decreased estradiol, increased FSH and increased LH levels.

In normal ovarian tissues surgically removed from young premenopausal patients with advanced cervical cancer treated preoperatively by CPT-11 combination chemotherapies, no growing ovarian follicles were detected histopathologically, and only a few non-growing primordial follicles were observed. These results suggest the hypothesis that CPT-11 may directly cause ovarian follicular loss. Ovarian follicular loss means the death of estrogen-producing granulosa cells that induces sufficient reduction of the serum estradiol level to secondarily increase the serum FSH and LH levels in a negative feedback mechanism. Even though only a few primordial follicles were detected in ovaries treated with CPT-11 within a few months, repeated CPT-11 injections may stimulate depletion of promising ovarian follicles that can ovulate and lead to pregnancy in the future. Finally, it is highly likely that high-dose and/or repeated CPT-11 injections induce severe dysovulation or premature ovarian failure in young women after chemotherapy. The normal ovaries examined histopathologically were surgically removed about 1 month after the last CPT-11 injection, suggesting that CPT-11-induced ovarian follicular loss can be induced within a short time. However, the present pathological results 1 month after CPT-11 injection do not show any ovarian changes immediately after CPT-11 injection that reveal possible molecular mechanisms to regulate ovarian follicular loss. Therefore, we recently investigated the mechanisms of CPT-11-induced ovarian follicular loss using *in vivo* animal experiments. These animal experiments revealed that CPT-11 induces stage-specific granulosa cell apoptosis via the Fas/Fas ligand cell death signaling system (25). We are currently developing a new protective method for anticancer drug-induced ovarian failure using *in vivo* animal experiments.

In conclusion, CPT-11 injections frequently deplete ovarian follicles to an extent that inhibits estrogen production. This effect induces MMLS in perimenopausal women aged ~50 years. Repeated CPT-11 injections to young women can cause severe ovarian damage within a short time, which is sufficient to induce severe sterility and premature ovarian failure. Endocrinological examinations revealed that injections of CPT-11 at therapeutic doses hardly affect the endocrine functions of the hypothalamus and pituitary gland, even though the ovaries are easily affected by these therapeutic CPT-11 injections. From these results, we propose that it is necessary to describe and explain the strong ovarian toxicity of CPT-11 before administering such chemotherapy to young cancer patients who are hoping for future pregnancies, and that CPT-11 combination chemotherapies should only be carried out on selected young women after informed consent is obtained.

Acknowledgments

This study was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan.

References

1. Sutcliffe SB: Cytotoxic chemotherapy and gonadal function in patients with Hodgkin's disease. Facts and thoughts. JAMA 242: 1898-1901, 1979.
2. Byrne J, Mulvihill JJ, Myers MH, Connelly RR, Naughton MD, Krauss MR, Steinhorn SC, Hassinger DD, Austin DF, Bragg K, *et al*: Effects of treatment on fertility in long-term survivors of childhood or adolescent cancer. N Engl J Med 317: 1315-1321, 1987.
3. Rivkees SA and Crawford JD: The regulationship of gonadal activity and chemotherapy-induced gonadal damage. JAMA 259: 2123-2125, 1988.
4. Chapman RM, Sutcliffe SB and Malpas JS: Male gonadal dysfunction: a prospective study. JAMA 245: 1323-1328, 1981.
5. Koyama H, Wada T, Nishizawa Y, Iwanaga T and Aoki Y: Cyclophosphamide-induced ovarian failure and its therapeutic significance in patients with breast cancer. Cancer 39: 1403-1409, 1977.
6. Horning SJ, Hoppe RT, Kaplan HS and Rosenberg SA: Female reproductive potential after treatment for Hodgkin's disease. N Engl J Med 304: 1377-1382, 1981.
7. Bakchine H, Brauner R, Thibaud E, Rappaport R, Flamant F, Griscelli C, Lemerle J, Schaison G, Schweisguth O and Zucker JM: Chemotherapy and ovarian function. Retrospective analyses in 17 girls treated for malignant tumor of hematologic disease. Arch Fr Pediatr 43: 611-616, 1986.
8. Bianco AR, Del Mastro L, Gallo C, Perrone F, Matano E, Pagliarulo C and De Placido S: Prognostic role of amenorrhea induced by adjuvant chemotherapy in premenopausal patients with early breast cancer. Br J Cancer 63: 799-803, 1991.
9. Chemaitilly W, Mertens AC, Mitby P, Whitton J, Stovall M, Yasui Y, Robison LL and Sklar CA: Acute ovarian failure in the childhood cancer survivor study. J Clin Endocrinol Metab 91: 1723-1728, 2006.
10. Sonmezer M and Oktay K: Fertility preservation in female patients. Hum Reprod Update 10: 251-266, 2004.
11. Wallace WH, Anderson RA and Irvine DS: Fertility preservation for young patients with cancer; who is at risk and what can be offered? Lancet Oncol 6: 209-218, 2005.
12. Kawato Y, Aonuma M, Hirota Y, Kuga H and Sato K: Intracellular roles of SN-38, a metabolite of the camptothecin derivative CPT-11, in the antitumor effect of CPT-11. Cancer Res 51: 4187-4192, 1991.

ma T, Yakushiji M, Noda K, Ikeda M, Kudoh R, Yajima A, da Y, Terashima Y, Takeuchi S, Hiura M, Saji F, Takahashi T, Umesaki N, Sato S, Hatae M and Ohashi Y: Phase II study of irinotecan and cisplatin as first-line chemotherapy in advanced or recurrent cervical cancer. Oncology 58: 31-37, 2000.
14. Umesaki N, Fujii T, Nishimura R, Tanaka T, Nishida M, Fushiki H, Takizawa K, Yamamoto K, Hasegawa K and Izumi R: Phase II study of irinotecan combined with mitomycin-C for advanced or recurrent squamous cell carcinoma of the uterine cervix: the JGOG study. Gynecol Oncol 95: 127-132, 2004.
15. Tanaka T, Kokawa K and Umesaki N: Preoperative chemotherapy with irinotecan and mitomycin for FIGO stage IIIb cervical squamous cell carcinoma: A pilot study. Eur J Gynaecol Oncol 26: 605-607, 2005.
16. Tanaka T, Umesaki N and Ogita S: Camptothecin and mitomycin combination chemotherapy on ovarian clear cell carcinoma with multiple systemic metastases. Eur J Gynaecol Oncol 21: 377-379, 2000.
17. Nishino K, Aoki Y, Amikura T, Obata H, Sekine M, Yahata T, Fujita K and Tanaka K: Irinotecan hydrochloride (CPT-11) and mitomycin C as the first line chemotherapy for ovarian clear cell adenocarcinoma. Gynecol Oncol 97: 893-897, 2005.
18. Noda K, Nishiwaki Y, Kawahara M, Negoro S, Sugiura T, Yokoyama A, Fukuoka M, Mori K, Watanabe K, Tamura T, Yamamoto S and Saijo N: Irinotecan plus cisplatin compared with etoposide plus cisplatin for extensive small-cell lung cancer. N Engl J Med 346: 85-91, 2002.
19. Saltz LB, Cox JV, Blanke C, Rosen LS, Fehrenbacher L, Moore MJ, Maroun JA, Ackland SP, Locker PK, Pirotta N, Elfring GL and Miller LL: Irinotecan plus fluorouracil and leucovorin for metastatic cororectal cancer. N Engl J Med 343: 905-914, 2000.
20. Douillard JY, Cunningham D, Roth AD, Navarro M, James RD, Karasek P, Jandik P, Iveson T, Carmichael J, Alaki M, Gruia G, Awad L and Rougier P: Irinotecan combined with fluorouracil compared with fluorouracil alone as first-line treatment for metastatic colorectal cancer: a multicentre randomized trial. Lancet 355: 1041-1047, 2000.
21. Yamao T, Shirao K, Matsumura Y, Muro K, Yamada Y, Goto M, Chin K and Shimada Y: Phase I-II study of irinotecan combined with mitomycin-C in patients with advanced gastric cancer. Ann Oncol 12: 1729-1735, 2001.
22. Tobinai K and Hotta T: Clinical trials for malignant lymphoma in Japan. Jpn J Clin Oncol 34: 369-378, 2004.
23. Itabashi M, Inoue T, Amano Y and Sato K: Reproduction and developmental toxicity studies of CPT-11. Clin Res 24: 85-96, 1990.
24. Tanaka T, Umesaki N and Ogita S: Effects of shakuyaku-kanzo-to on menopausal symptoms in an ovarian cancer patient treated with irinotecan HCI. J Trad Med 15: 456-457, 1998.
25. Utsunomiya T, Tanaka T, Utsunomiya H and Umesaki N: A novel molecular mechanism for anticancer drug-induced ovarian failure: irinotecan HCl, an anticancer topoisomerase I inhibitor, induces specific FasL expression in granulosa cells of large ovarian follicles to enhance follicular apoptosis. Int J Oncol 32: 991-1000, 2008.