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7-Ethyl-10-[4-(1-piperidino)-1-piperidino] carbonyloxy camptothecin: mechanism of resistance and clinical trials

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Abstract. The camptothecin derivative 7-ethyl-10-[4-(1-piperidino)-1-piperidino]-carbonyloxy camptothecin (CPT-11) has attracted the attention of clinicians because of its high antitumor activity against refractory solid cancers. We established two CPT-11-resistant cell lines, a non-small-cell lung-cancer cell line (PC-7/CPT-11) from the parental PC-7 line and an ovarian cancer cell line (HAC-2/CPT-11) from the parental HAC-2 line. The mechanisms of resistance to CPT-11 in PC-7/CPT-11 cells were reduced conversion of CPT-11 to its active metabolite SN-38 and point mutation of topoisomerase I. Those in HAC-2/CPT-11 cells were reduction of topoisomerase I activity and decreased sensitivity of topoisomerase to topoisomerase I inhibitors. No point mutation of the topoisomerase was observed in HAC-2/CPT-11 cells. We conducted two phase I trials using CPT-11 in combination with other anticancer agents. One was a phase I trial of CPT-11 and cisplatin given with a fixed dose of vindesine to patients with advanced non-small-cell lung-cancer and the other was a phase I study on a topoisomerase-targeting combination of CPT-11 and etoposide (VP-16) in patients with various malignant solid tumors. The results of the first trial indicated that the recommended dose of CPT-11 for phase II studies was 80 mg/m^2 combined with 3 mg/m^2 vindesine on days 1 and 8 and 60 mg/m^2 cisplatin on day 1. In the second trial, the recommended dose of CPT-11/VP-16 given with recombinant granulocyte colony-stimulating factor (on days 4–17) was found to be 60/60 mg/m^2. In both trials, diarrhea and granulocytopenia were considered to be dose-limiting toxicities.

Key words: CPT-11 – Topoisomerase I – Dose-limiting toxicity – Resistance

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Introduction

A great deal of attention has been paid to DNA topoisomerases as targets for cancer chemotherapy. The DNA topoisomerases are essential nuclear enzymes for cell division and growth and have been classified into two types, I and II [6, 11, 16, 31]. They catalyze changes in the topological state of duplex DNA by performing single- and double-strand breakage-resealing cycles [28].

Camptothecin is an alkaloid antitumor agent, which was isolated from *Camptotheca acuminata*, a tree native to South China. Although camptothecin itself has been reported to demonstrate antitumor activity against various experimental tumors, it had severe adverse effects and its clinical use was abandoned [7]. Efforts have been directed to the synthesis of new camptothecin derivatives with higher antitumor activity and fewer adverse effects. The Yakult Company (Tokyo) succeeded in developing 7-ethyl-10-[4-(1-piperidino)-1-piperidino]-carbonyloxy camptothecin (CPT-11) [7, 15, 23]. Early clinical trials demonstrated that it had significant antitumor activity against refractory solid tumors such as non-small-cell lung, stomach, colonic, and other cancers [4, 26, 27, 29]. The side effects of CPT-11 observed in phase I and II studies were controllable.

For the successful use of this agent in practice, it is important that the mechanisms of tumor resistance to CPT-11 be elucidated. We established CPT-11-resistant human lung and ovarian cancer cell lines and characterized them. We also conducted two phase I trials of CPT-11 given in combination both with cisplatin and vindesine and with cisplatin and etoposide (VP-16) and decided the recommended dose regimens for future clinical trials.

Establishment and characterization of a CPT-11-resistant human non-small-cell lung-cancer cell line

The CPT-11-resistant non-small-cell lung-cancer (NSCLC) cell line PC-7/CPT-11 was established by stepwise and continuous exposure of the parental PC-7 cell line to CPT-11, the respective starting and final concentrations of which

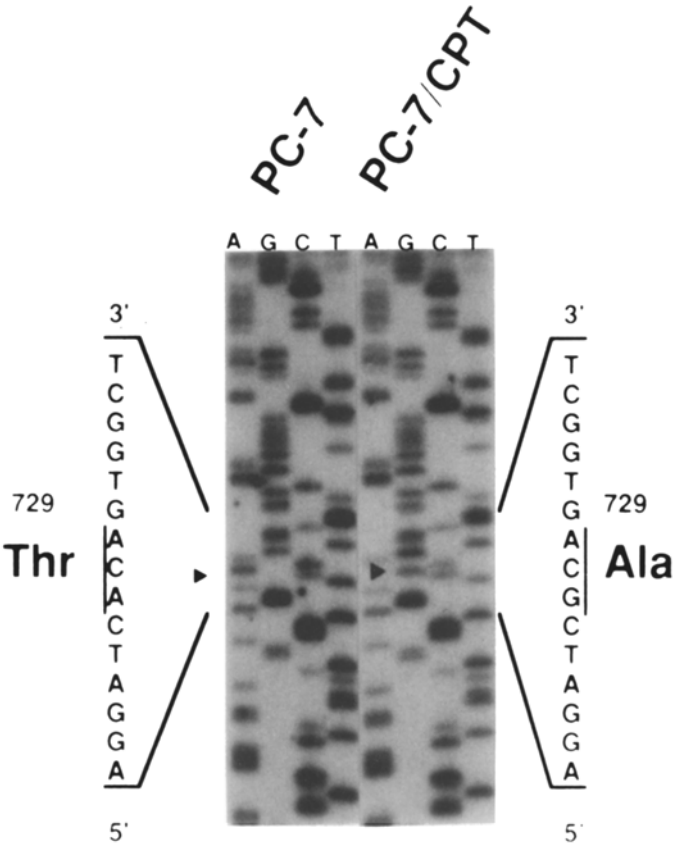

Fig. 1. Replacement of the conserved threonine (*Thr*) at position 729 of the topoisomerase I gene in PC-7 with alanine (*Ala*) in the PC-7/CPT-11

were 0.1 and 0.5 μg/ml. The PC-7/CPT-11 cells grew more slowly than the PC-7 cells (doubling time, 45.8 versus 35.5 h), and their plating efficiency (3.2%) was lower than that of PC-7 cells (7.1%). The 50% growth-inhibitory concentrations (IC_{50} values) of CPT-11 for PC-7 and PC-7/CPT-11 cells were 0.037 and 0.35 μg/ml, respectively. The PC-7/CPT-11 cell line showed 9.5-fold resistance to CPT-11 as compared with the PC-7 line. It also showed cross-resistance to SN-38, an active metabolite of CPT-11, but not to other anticancer drugs. The intracellular accumulation of CPT-11 in these two cell lines did not differ, but the intracellular formation of SN-38 by PC-7/CPT-11 was significantly lower. The topoisomerase I activity of PC-7/CPT-11 cells was about one-fourth that of PC-7 cells, and the enzyme isolated from the parental PC-7 cells was at least 5 times more sensitive to CPT-11 than that isolated from the PC-7/CPT-11 cells [8]. We speculated that an alteration of the topoisomerase I gene may have been responsible for the change in topoisomerase activity observed in the PC-7/CPT-11 line.

Therefore, we analyzed the topoisomerase I gene (Topo I) mutation using the single-strand conformation polymorphism-polymerase chain reaction with reverse transcriptase (SSCP-PCR)method. We divided the Topo I cDNA into ten fragments that overlapped each other and covered all the coding sequences of the Topo I cDNA. We observed a mobility shift of two fragments from PC-7/CPT-11, which suggested the presence of some mutations in these fragments. We carried out direct sequencing of these portions using the dideoxy chain-termination method and observed an altered sequence with a G- to A-base change in PC-7/CPT-11 as compared with PC-7. This base substitution resulted in replacement of the conserved threonine at position 729 of PC-7 with alanine (Fig. 1) [14]. These results suggest that the Topo I gene-point mutation is related to the decreased topoisomerase I activity and reduced sensitivity to topoisomerase I inhibitors of PC-7/CPT cells.

Establishment and characterization of a CPT-11-resistant ovarian cancer cell line

A CPT-11-resistant human ovarian cell line, HAC-2/CPT-11, was established from parental HAC-2 cells. The diameters, cloning efficiency, and doubling time of HAC-2/CPT-11 cells were almost same as those of HAC-2. The HAC-2/CPT-11 cell line showed 9.7- and 4.7-fold resistance to CPT-11 and SN-38, respectively, as compared with HAC-2 but showed no cross-resistance against VP-16, Adriamycin, cisplatin, taxol, actinomycin D, and vincristine, which suggests that HAC-2/CPT-11 had acquired specific resistance to topoisomerase inhibitors. The topoisomerase I activity of HAC-2/CPT-11 was about half that of HAC-2, and SSCP-PCR analysis demonstrated no point mutation in the topoisomerase I cDNA from HAC-2/CPT-11. The amount of topoisomerase I protein detected in HAC-2/CPT-11 was lower than that found in HAC-2. These results suggest that the lower topoisomerase I protein content causes the reduced topoisomerase I activity and the decreased sensitivity to topoisomerase I inhibitors of HAC-2/CPT-11 as compared with HAC-2.

A disease-oriented phase I clinical trial of CPT-11 and cisplatin in combination with vindesine in patients with advanced NSCLC

The outcome for patients with advanced NSCLC remains miserable, and only six drugs appear to have moderate activity against it: cisplatin, ifosfamide, mitomycin C, vindesine, etoposide, and vinblastine. Some data demonstrate that cisplatin-based combination chemotherapy may produce a significant survival benefit, even in patients with stage IV NSCLC. However, it would appear that advances in the treatment of this disease will be achieved only by discovering new active agents. In a phase II trial of CPT-11 against NSCLC, the response rate was found to be 32% on an i.v. administration schedule of 100 mg/m^2 weekly.

We conducted phase I trials of cisplatin, vindesine, and CPT-11 combination therapy (1) to determine the optimal doses of CPT-11 given in combination with cisplatin and a fixed dose of vindesine and (2) to evaluate the toxicity of CPT-11 in combination chemotherapy. A total of 26 patients with stage IV NSCLC were accrued from July 1991 to December 1992. Patients with histologically or cytologically confirmed metastatic NSCLC were eligible for entry into this phase I trial. None of them had had prior chemotherapy and/or radiotherapy. The other eligibility

Table 1. Toxicities encountered in a phase I clinical trial of CPT-11 and cisplatin in combination with vindesine

Dose (mg/m²)		Number of patients/ number of cycles/ number of patients with DLT	Common toxicity grade (number of patients)														
CPT-11	Cisplatin		WBC			Granulocytes			Platelets			Diarrhea		Nausea/ vomiting		Liver		Kidney
			2	3	4	2	3	4	2	3	4	2	≧3	2	3	2	3	≧2
20	60	3/9/0	2	1	0	0	1	2	0	0	0	0	0	2	0	0	0	0
40	60	3/13/0	1	1	1	0	2	1	0	0	0	1	0	2	0	0	0	0
60	60	3/7/0	1	1	1	1	0	2	0	0	0	1	0	2	0	0	0	0
60	80	5/11/3	1	4	0	0	0	5 (1)[a]	0	0	0	0	2	2	2	0	0	0
80	60	7/15/2	2	2	3	0	1	6	1	0	0	2	1	3	2	0	1	0
100	60	5/7/3	1	2	2	0	1	4 (2)[a]	0	1	0	1	2	4	0	0	1	0

DLT, Dose-limiting toxicity

[a] Number of patients who experienced grade 4 granulocytopenia lasting for ≧7 days

criteria comprised an expected survival of 6 weeks, an age of less than 75 years, an Eastern Cooperative Oncology Group (ECOG) performance score of 0–1, measurable lesions, no brain metastasis, adequate hematological [white blood cell count (WBC) 4,000/mm³; platelet count, 100,000/mm³; hemoglobin, 11 g/dl], renal (serum creatinine, 1.5 mg/dl; creatinine clearance, 60 ml/min), and hepatic function [total serum bilirubin, 1.5 mg/dl; SGOT and SGPT levels of less than twice the normal range; indocyanine green (ICG) test, 15%].

A sequential cohort of patients was used to study the interpatient dose escalation of CPT-11 (days 1 and 8) and cisplatin (day 1) given with 3 mg/m² vindesine (days 1 and 8). The starting doses of CPT-11 and cisplatin were 20 and 60 mg/m², respectively, and the doses of both agents were escalated in increments of 20 mg/m². The use of granulocyte colony-stimulating factor (G-CSF) was not allowed in this trial. The maximum tolerated dose (MTD) was regarded as having been reached when any of the following criteria had been fulfilled in at least 50% of the patients treated: (1) grade 3 or greater nonhematological toxicity and/or grade 4 nausea and vomiting and (2) the persistence of an absolute granulocyte count nadir of 500 cells/mm³ and/or a platelet count nadir of 50,000/mm³ for 7 days or more. These hematological parameters were determined by checking the complete blood counts every other day when the leukocyte and platelet counts had declined to below 2,000/mm³ and 50,000/mm³, respectively.

The toxicities encountered in this trial are summarized in Table 1. Grade 4 granulocytopenia was observed at each dose level. At the fourth level (CPT-11, 60 mg/m²; cisplatin, 80 mg/m²), all five patients treated developed grade 4 granulocytopenia, which lasted for 9 days in one case, and they all experienced grade 3 or 4 diarrhea. At the fifth level (CPT-11,80 mg/m²; cisplatin, 60 mg/m²), six of the seven patients treated developed grade 4 granulocytopenia, which in no case lasted for more than 7 days, and diarrhea and elevation of transaminases (both grade 3) occurred in one patient. During treatment with CPT-11 at 100 mg/m² and cisplatin at 60 mg/m², one patient developed grade 4 granulocytopenia lasting for 7 days and grade 3 diarrhea; one experienced grade 4 granulocytopenia lasting for 7 days, grade 4 fever, and grade 4 diarrhea; and one exhibited grade 3 transaminase and bilirubin elevations (Table 1). Thrombocytopenia was generally mild during all dose regimens.

For the combination of CPT-11, cisplatin, and vindesine (3 mg/m² on days 1 and 8), the MTD of CPT-11 combined with cisplatin (60 mg/m² on day 2) was 80 mg/m² on days 1 and 8 [30]. Of the 22 evaluable patients, 9 achieved a partial response.

A phase I study of topoisomerase-targeting chemotherapy: CPT-11 plus VP-16

Several investigators have reported a lack of cross-resistance to the camptothecins and topoisomerase II inhibitors. In the majority of topoisomerase I inhibitor-resistant cell lines, the sensitivity to topoisomerase II inhibitors is increased. Furthermore, the results of some preclinical studies suggest that CPT-11 treatment enhances the antitumor activity of several anticancer agents, including topoisomerase II inhibitors. The combination of topoisomerase I and II inhibitors appears to be an extremely attractive strategy for cancer chemotherapy because of their complementary functions. Therefore, we conducted a phase I study of CPT-11 and VP-16 given in combination as topoisomerase I/II-targeting chemotherapy. The aims of this study were (1) to determine the maximum tolerated and acceptable doses of CPT-11 and VP-16, which were given in combination with G-CSF; (2) to observe and document any toxicity occurring with this combination; and (3) to observe the therapeutic activity of this regimen.

A total of 34 patients admitted to the National Cancer Center Hospital from July 1991 to October 1992 were entered into this study on the basis of the following eligibility criteria: (1) a histologically or cytologically proven malignant solid tumor; (2) disease that had not been treated with or had failed standard chemotherapy; (3) an age of less than 75 years; (4) a life expectancy of at least 8 weeks; (5) a performance status of 2 or better on the ECOG scale (ambulatory and capable of self-care); (6) no previous chemotherapy or radiotherapy within 4 weeks prior to entry; (7) adequate renal (serum creatinine, 1.5 mg/ml; creatinine clearance, 30 ml/min), hepatic (total bilirubin, 1.5 mg/dl; transaminase levels, 2× the upper limit of normal), and bone marrow function (leukocyte count, 3,000/

Table 2. Toxicities encountered in a combination phase I study of CPT-11 and VP-16

Level	CPT-11/VP-16/GCSF dose (mg/m^2)	Number of patients (courses)	Number of nointolerating patients	Diarrhea (grade 3+4)	Grade 4 granulocytopenia	
					Total	>4 days
I	40/60/–	2 (3)	2	0	2	2
I	40/60/+	6 (8)	2	1	1	1
II	60/60/+	13 (36)	2	1	3	2
IIa	60/80/+	6 (16)	4	3	3	2
IIb	80/60/+	5 (11)	3	3	3	1

IIa, Dose in mg/m^2 on days 1–3; IIb, 50 μg/m^2 on days 4–17

mm^3, platelet count, 100,000/mm^3; hemoglobin concentration, 9.0 g/dl); (8) no other coexisting medical problem of sufficient severity to prevent full compliance with the study; and (9) the acquisition of informed consent prior to the start of treatment.

The starting doses of CPT-11 and VP-16 were 40 mg/m^2 given on days 1–3 and 60 mg/m^2 given on days 1–3, respectively. The dose levels of CPT-11/VP-16 given on days 1–3 were escalated to 40/60, 60/60, 60/80, and 80/60 mg/m^2. The VP-16 was dissolved in 100 ml normal saline and given as a 60-min infusion, immediately after which CPT-11 (dissolved in 250 ml 5% fructose) was given as a 90-min i.v. infusion. Both drugs were delivered using an electric infusion pump. Because either grade 3 leukopenia continuing for more than 8 days or grade 4 granulocytopenia of more than 4 days' duration was observed in the first two patients receiving the lowest dose of CPT-11 and VP-16, G-CSF (50 μg/m^2 on days 4–17) was injected s.c. in all the patients. We considered drug toxicities to be intolerable if patients experienced one or more of the following: (1) grade 4 granulocytopenia lasting for 4 days, (2) grade 3 leukopenia lasting for 8 days, and (3) grade 3 toxicity in a specific organ system. The dose was considered to be the MTD if two-thirds of the patients treated experienced intolerable adverse effects. Anemia, alopecia, and nausea and vomiting were excluded from the MTD evaluation.

Gastrointestinal (GI) toxicity was observed with all the regimens, even at the starting drug levels, and the signs and symptoms comprised nausea, vomiting, loss of appetite, body weight loss, and various degrees of diarrhea. The severity of these adverse effects appeared in general to be dose-related. We used metoclopramide, domperidone, or promethazine HCl, but not dexamethasone, as antiemetics during the first course of treatment so as to observe the associated adverse effects precisely. Nausea and appetite loss were severe and many patients lost weight; the median body weight loss was 5%. All the patients entered at levels IIIa and IIIb suffered from diarrhea and abdominal pain. Level IIIa fulfilled our MTD criteria. We interrupted treatment during the level IIIb regimen because it caused severe diarrhea. Alopecia was common and reversible, and we found no evidence of pulmonary toxicity or cystitis. During the second course of treatment, we used dexamethasone (8 mg/patient given on days 1–3) with or without granisetron (4,000 μg/patient given on days 1–3) as an antiemetic. The GI toxicity and hematotoxicity appeared to be less severe than those encountered during the first course.

Granulocytopenia was so severe that this regimen required supportive therapy with G-CSF (Table 2), and there was a trend toward increasing severity of granulocytopenia as the dose levels increased. Thrombocytopenia occurred less frequently than granulocytopenia and was generally mild. From the toxicity results, it was decided that level II was tolerable.

Patients were considered evaluable for therapeutic efficacy if they had received at least three cycles of treatment or experienced progressive disease. In all, 22 patients were evaluable. Five of the previously untreated NSCLC patients achieved a partial response (PR), as did two patients with NSCLC who had received chemotherapy previously, two patients with head and neck cancer, and one patient with an adenocarcinoma of unknown primary origin. At level II, 5 of the 11 evaluable patients achieved PRs: 2 patients with untreated NSCLC, 1 patient with NSCLC who had received previous chemotherapy and 1 patient with head and neck cancer. Therefore, we concluded that level II (CPT-11/VP-16 given at 60/60 mg/m^2 on days 1–3) would be appropriate for phase II trials [9].

Discussion

Drug resistance may be due to decreased uptake of drugs, accelerated drug removal by P-glycoprotein, drug detoxification by increased levels of cellular glutathione or metallothionein, enhanced DNA repair, gene amplification and subsequent overexpression of the target molecule, or decreased levels of target enzymes [2, 3, 12, 14, 17, 18, 20, 21, 25]. In this study, we analyzed the mechanisms of CPT-11 resistance in PC-7/CPT-11 and HAC-2 cell lines in vitro (Table 3).

In PC-7/CPT-11 cells, the low ability to form intracellular SN-38 would appear to be a mechanism of CPT-11 resistance [24]. The total activity of DNA topoisomerase I and the amount of topoisomerase I protein appeared to be lower in PC-7/CPT-11 cells than in the parent cells. The DNA topoisomerase I extracted from PC-7/CPT-11 cells was at least 5 times more resistant to the inhibitory effect of topoisomerase I than was that extracted from the parent cells. Topoisomerase I cDNA from the PC-7/CPT line contained an altered sequence with a G- to A-base change; this base substitution resulted in the replacement of the threonine at position 729 of PC-7 with an alanine in the C-terminal position of the protein. In HAC-2/CPT-11 cells, the activity and the amount of topoisomerase I were lower

Table 3. Characteristics of CPT-resistant cell lines

Cell line	Selecting agent	Origin	Relative resistance	Topo I level	CPT sensitivity of Topo I	Gene alteration	Topo I uptake	References
PC-7/CPT-11	CPT-11	Human lung cancer	9	0.25	R	+ mutation	→	Kanzawa et al. [8]
HAC-2/CPT-11	CPT-11	Human ovarian cancer	9.7	0.5	0.5	–		Kubota et al. [14]
CPT-K5	CPT-11	Human TALL	300	0.3–0.5	R	+ mutation		Andoh et al. [1]
CPT-B	CPT	CHO	250–350	0.4–0.5	R	ND		Gupta et al. [5]
HT-29/CPT	CPT	Human colon cancer	7	0.13	S	+ deletion		Sugimoto et al. [30a]
A549/CPT	CPT	Human lung cancer	2	1.0	ND	ND		Sugimoto et al. [30a]
St-4/CPT	CPT	Human gastric cancer	9	0.25	ND	ND		Sugimoto et al. [30a]
DC3F/C10	CPT	CHL	134	1.0	R	+ mutation		Pommier et al. [28a]
V79	CPT	CHL	14	0.25	ND			Chang et al. [2a]
IRS-1	CPT	CHL	2	0.1	S		↓	Chang et al. [2a]
IRS-2	CPT	CHL	34	0.5	R		↓	Chang et al. [2a]

CHO, Chinese hamster ovary cell; CHL, Chinese hamster lung cell; Topo I, topoisomerase I; R, resistant; S, sensitive; ND, not determined

than those of its parent, but no topoisomerase I mutation was detected. Several studies on camptothecin- and CPT-11-resistant cell lines have been carried out, the majority of which showed point mutation or deletion of the topoisomerase I gene [1, 5]. These resistant cell lines serve as important tools for the selection of new anticancer agents that can overcome CPT-11 resistance.

The intractable diarrhea induced by CPT-11 was dose-limiting. In the phase I study of cisplatin, vindesine, and CPT-11 given in combination, fewer episodes of diarrhea were observed when low-dose cisplatin was used. The diarrhea induced by CPT-11 was resistant to standard doses of antidiarrheal drugs such as loperamide HCl and opium tincture. The mechanism responsible is unclear, although, there is a positive correlation between the area under the concentration-time curve (AUC) and peak concentration (Cmax) of SN-38 and the incidence and/or severity of diarrhea episodes. In our experience, however, 40 μg/kg granisetron (i.v.) given twice a day for 6 days from the initiation of chemotherapy and maintenance of the granulocyte count level with G-CSF can reduce the incidence and/or severity of the diarrhea induced by CPT-11 and cisplatin [22] For the combination of CPT-11 and cisplatin given without vindesine, Fukuoka et al. [4] reported that the phase II doses of each drug were 60 mg/m^2 weekly and 80 mg/m^2 every 3 weeks, respectively, and demonstrated a 47.5% PR rate in patients with untreated NSCLC using this regimen.

In our phase I study of topoisomerase-targeting chemotherapy using CPT-11 and VP-16, the dose-limiting toxicities were profound granulocytopenia and diarrhea. Although the recommended CPT-11/VP- 16 dose for phase II trials was 60/60 mg/m^2 given on days 1–3 every 3 weeks, supportive therapy with G-CSF on days 4–17 was essential. Furthermore, these adverse effects appeared to be reduced by dexamethasone and granisetron. The Japanese Clinical Oncology Group (JCOG) has just started a phase II study of this combination chemotherapy in patients with NSCLC who have received no previous chemotherapy. The limiting sampling strategy for evaluation of the drug-drug interaction has just been established [10], and we shall evaluate the validity of this strategy prospectively.

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References

1. Andoh T, Ishii K, Suzuki Y, Ikegami Y, Kusunoki Y, Takemoto Y, Okada K (1987) Characterization of a mammalian mutant with a camptothecin-resistant DNA topoisomerase I. Proc Natl Acad Sci USA 84: 5565–5569
2. Bungo M, Fujiwara Y, Kasahara K, Nakagawa K, Ohe Y, Sasaki Y, Irino S, Saijo N (1990) Decreased accumulation as a mechanism of resistance to *cis*-diamminedichloroplatinum(II) in human non-small cell lung cancer cell line; relation to DNA damage and repair. Cancer Res 50: 25049–2553
3. Fujiwara Y, Kasahara K, Sugimoto Y, Nishio K, Ohmori T, Saijo N (1990) Detection of proteins that recognize platinum-modified DNA using gel mobility shift assay. Jpn J Cancer Res 81: 1210–1213
4. Fukuoka M, Niitani H, Suzuki S, Motomiya M, Hasegawa K, Nishiwaki Y, Kuriyama T, Ariyoshi Y, Negoro S, Masuda N, Nakajima S, Taguchi T (1992) A phase II study of CPT-11, a new derivative of camptothecin, for previously untreated non-small cell lung cancer. J Clin Oncol 10: 16–20
5. Gupta RS, Gupta R, Eng B, Lock RB, Ross WE, Hertzberg RR, Caranga MJ, Johnson RK (1988) Camptothecin-resistant mutants of Chinese hamster ovary cells containing a resistant form of topoisomerase I. Cancer Res 48: 6404–6410
6. Hsiang YH, Hertzberg R, Hecht S, Liu LF (1985) Camptothecin induces protein-linked DNA breaks via mammalian DNA topoisomerase I. J Biol Chem 260: 14873–14875
7. Kaneda N, Nagata H, Furuta T, Yokokura T (1990) Metabolism and pharmacokinetics of the camptothecin analogue CPT-11 in the mouse. Cancer Res 50: 1715–1720
8. Kanzawa F, Sugimoto Y, Minato K, Kasahara K, Bungo M, Nakagawa K, Fujiwara Y, Liu LF, Saijo N (1990) Establishment of a camptothecin analogue (CPT-11)-resistant cell line of human non small cell lung cancer: characterization and mechanism of resistance. Cancer Res 50: 5919–5924
9. Karato A, Sasaki Y, Shinkai T, Eguchi K, Tamura T, Ohe Y, Oshita F, Nishio M, Kunikane H, Arioka H, Ohmatsu H, Nakashima H, Shiraishi J, Saijo N (1993) A phase I study of CPT-11 and VP-16. J Clin Oncol 11: 2030–2035
10. Karato A, Nakashima H, Sasaki Y, Shiraishi J, Ohmatsu H, Oshita F, Atrioka H, Eguchi K, Kunikane H, Nishio M, Ohe Y, Tamura T, Shinkai T, Saijo N (1993) Pharmacokinetic analysis of the combination of CPT-11 and VP-16. Proc Jpn Cancer Assoc 52: 2141

11. Kojima A, Shinkai T, Saijo N (1993) Cytogenetic effects of CPT-11 and its active metabolite, SN-38, on human lymphocytes. Jpn J Clin Oncol 23: 116–122
12. Kasahara K, Fujiwara Y, Nishio K, Ohmori T, Sugimoto Y, Komiya K, Matsuda T, Saijo N (1991) Metallothionein content correlates with the sensitivity of human small cell lung cancer cell lines to cisplatin. Cancer Res 51: 3237–3242
13. Kijima T, Kubota N, Nishio K (1994) Establishment of a CPT-11 resistant human ovarian cell line. Anticancer Res. (in press)
14. Kubota N, Kanzawa F, Nishio K, Takeda Y, Ohmori T, Fujiwaya Y, Terashima Y, Saijo N (1992) Detection of topoisomerase I gene point mutation in CPT-11 resistant lung cancer cell line. Biochem Biophys Res Commun 188: 571–577
15. Kunimoto T, Nitta K, Tanaka T, Uehara N, Baba H, Takeuchi M, Yokokura T, Sawada S, Miysaka T, Mukai M (1987) Antitumor activity of 7-ethyl-10-[4-(1-piperidino)-1-piperidino]-carbonyloxy-camptothecin, a novel water-soluble derivative of camptothecin, against murine tumors. Cancer Res 47: 5944–5947
16. Liu LF, Miller KG (1981) Eukaryotic DNA topoisomerases: two forms of type I DNA topoisomerases from HeLa cell nuclei. Proc Natl Acad Sci USA 78: 3487–3491
17. Marikage T, Ohmori T, Nishio K, Fujiwara Y, Takeda Y, Saijo N (1993) Modulation of cisplatin sensitivity and accumulation by amphotericin B in cisplatin resistant human lung cancer cell line. Cancer Res 53: 3302–3307
18. Minato K, Kanzawa F, Nakagawa K, Sugimoto Y, Fujiwara Y, Nishio K, Saijo N (1990) Characterization of an etoposide-resistant human small cell lung cancer cell line. Cancer Chemother Pharmacol 26: 313–317
19. Muggia FM, Creaven PJ, Hansen HH, Cohen, MH, Selawry OS (1972) Phase I clinical trial of weekly and daily treatment with camptothecin (NSC-100880): correlation with preclinical studies. Cancer Chemother Rep 56: 515–521
20. Nakagawa K, Yokota J, Wada M, Sasaki Y, Fujiwara T, Tsunokawa Y, Terada M, Saijo N (1988) Levels of glutathione S transferase π mRNA in human lung cancer cell lines correlate with the resistance to cisplatin and carboplatin. Jpn J Cancer Res 79: 301–304
21. Nakagawa K, Saijo N, Tsuchida S, Sakai M, Tsunokawa Y, Yokota J, Muramatsu M, Sato K, Terada M, Tew KD (1990) Glutathione-S-transferase as a determinant of drug resistance in transfectant cell line. J Biol Chem 265: 4296–4301
22. Nakagawa K, Fukuoka M, Niitani H (1993) Phase II study of CPT-11 and cisplatin in patients with advanced non-small cell lung cancer. Proc Amer Soc Clin Oncol 12: 1104
23. Negoro S, Fukuoka M, Masuda N, Takada M, Kusunoki Y, Matsui K, Takafuji N, Kudoh S, Niitani H, Taguchi T (1991) Phase I study of weekly intravenous infusions of CPT-11, a new derivative of camptothecin, in a treatment of advanced non-small cell lung cancer. J Natl Cancer Inst 83: 1164–1168
24. Niimi S, Nakagawa K, Sugimoto Y, Nishio K, Fujiwara Y, Yokoyama S, Terashima Y, Saijo N (1992) Mechanism of cross-resistance to camptothecin analogue (CPT-11) in a human ovarian cancer cell line selected by cisplatin Cancer Res 52: 328–333
25. Nishio M, Arioka H, Nishio K, Kubota N, Heike Y, Ohata M, Saijo N (1993) Overexpression of *cis*-diamminedichloroplatinum (II)-inducible proteins that are recognized by anti-HMG antibody in cisplatin resistant human lung cancer cell lines. Proc Jpn Cancer Assoc 52: 2105
26. Ohe Y, Sasaki Y, Shinkai T, Eguchi K, Tamura T, Kojima K, Kunikane H, Okamoto H, Karato A, Ohmatsu H, Kanzawa F, Saijo N (1992) Phase I study and pharmacokinetics of CPT-11 with 5-day continuous infusion. J Natl Cancer Inst 84: 972–974
27. Ohno R, Okada K, Masaoka T, Kuramoto A, Arima T, Yoshida Y, Ariyoshi H, Ichimaru M, Sakai Y,Ito Y, Morishima Y, Yokomaku S, Ota K (1990) An early phase II study of CPT-11; a new derivative of camptothecin, for the treatment of leukemia and lymphoma. J Clin Oncol 8: 1907–1912
28. Pommier Y (1993) DNA topoisomerase I and II in cancer chemotherapy: update and perspectives. Cancer Chemother Pharmacol 32: 103–108
29. Shimada Y, Yoshino M, Wakui A, Nakao K, Futatsuki K, Sakata Y, Kambe M, Taguchi T (1993) Phase II study of CPT-11, a new camptothecin derivative, in patients with metastatic colorectal cancer. J Clin Oncol 11: 909–913
30. Shinkai T, Arioka H, Kunikane H, Eguchi K, Sasaki Y, Tamura T, Ohe Y, Nishio M, Karato A, Okamoto H, Nakashima H, Ohmatsu H, Shiraishi J, Saijo N (1994) A phase I clinical trial of CPT-11, 7-ethyl-10-[4-(1-piperidino)-1-piperidino]-carbonyloxy camptothecin, and cisplatin in combination with fixed dose of vindesine in advanced non-small cell lung cancer. Cancer Res (in press)
31. Wall ME, Wani MC, Cook CE, Palmar KH, Macphail AT, Simm GA (1966) Plant antitumor agents 1. The isolation and structure of camptothecin, a novel alkaloid leukemia and tumor inhibition from *Camptotheca acuminata*. J Am Chem Soc 88: 3888–3890