Anti-Cancer Drugs 1995, **6**, pp. 413–418

Cytotoxicity of CPT-11 for gastrointestinal cancer cells cultured on fixed-contact-sensitive plates

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The cytotoxicity of SN-38, the major metabolite of CPT-11 (7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecin, was compared among gastrointestinal carcinomas of every organ, and between primary and metastatic lesions of every organ-originated gastrointestinal carcinoma, by an *in vitro* anticancer drug sensitivity test using fixed-contact-sensitive plates. The rates of cases having a high response (percent survival 75% or lower) to SN-38 but a low response (percent survival above 75%) to cisplatin, mitomycin C (MMC), adriamycin (ADM) and 5-fluorouracil (5-FU) were 14.6, 19.4, 15.6 and 27.0%, respectively. While, the rates of cases having a high response to cisplatin, MMC, ADM and 5-FU but a low response to SN-38 were 7.3, 2.8, 9.4 and 13.5%, respectively. Each of the former rates were higher than each of the latter rates. In particular, the former rate for MMC was significantly higher than the latter rate ($p = 0.04$). Two cases with colon cancer showed a high response only to SN-38. The percent survival of primary lesions in colon cancer was significantly lower than that in stomach cancer. The rates of hepatocellular carcinoma cases having a high response to SN-38 but a low response to cisplatin, MMC, ADM and 5-FU were 16.7, 16.7, 0 and 25%, respectively. Only one case had a high response to 5-FU but a low response to SN-38. The percent survival of metastatic lesions in pancreatic cancer was significantly lower than that of primary lesions. From this study, we recommend the further clinical trial of CPT-11 for colon and hepato-cellular cancers.

Introduction

CPT-11 (7-ethyl-10-[4-(1-piperidino)-1-piperidino] carbonyloxycamptothecin) is a water-soluble analog of camptothecin synthesized by Yokokura and co-workers at Yakult Central Institute (Tokyo, Japan) in an attempt to identify derivatives with greater aqueous solubility and antitumor activity than CPT.[1] CPT-11 is a pro-drug that undergoes de-esterification *in vivo* to yield SN-38, a major metabolite that is 1000-fold more potent than the parent compound *in vitro*.[2,3] CPT-11 shows little inherent antitumor activity *in vitro*, but substantial activity against a variety of human tumor xenografts, including colon, mammary, gastric and squamous cell lung carcinomas implanted subcutaneously into nude mice.[4] Antitumor activity of CPT-11 has also been noted in clinical studies with various administration schedules.[5]

We previously established an *in vitro* assay system for predicting the drug responsiveness of primary cultured cells.[6] Superinoculation of neoplastic and normal cells onto confluent monolayers of a contact-sensitive cell line resulted in growth of the neoplastic cells and growth inhibition of the contact-sensitive normal cells. In the present study, we examined the cytotoxicity of CPT-11 for gastrointestinal cancer cells primarily cultured from patients by using this *in vitro* assay.

Materials and methods

Chemicals and Reagents

Dulbecco's modified Eagle's medium (DMEM) was purchased from Nissui Pharmaceutical (Japan). Fetal bovine serum (FBS) was obtained from Whittaker MA Bioproducts (USA). Sodium piperacillin, gentamicin and amphoterincin B were purchased from Toyama Chemical (Japan), Shering (Japan) and ER Squibb & Sons (Japan), respectively. [methyl-1,2-^{3}H]thymidine ([^{3}H]dThd) (specific activity: 120 Ci mmol) and ACS-II liquid scintillator were purchased from Amersham (France). Type 1 collagenase was obtained from Sigma (St Louis, MO). Trichloroacetic acid (TCA) was from Wako Pure Chemical (Japan). CPT-11 and SN-38 were gifts from Daiichi Pharmaceutical (Japan). Mitomycin C (MMC) and adriamycin (ADM) were gifts from Kyowa Hakko Kougyou (Japan). Cisplatin was a gift from Nippon Kayaku (Japan).

This work was supported by a grant-in-aid for cancer research from the Fukuoka Cancer Society

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Cell culture

BALB/c 3T3 A31 cells were originally isolated by Kakunaga;[7] these cells were recloned in our laboratory and found to be strictly sensitive to density-dependent growth inhibition. The KP-1N adenocarcinoma cell line, derived from human pancreas cancer, was established by Akira Kono.[8] The MKN-45 cell line,[9] isolated from poorly differentiated adenocarcinomas of human stomach cancer, was purchased from Immunobiological Laboratories.

These cell lines were maintained in 10% DMEM in a humidified atmosphere of 5% CO_2/95% air. The pH of the culture medium was maintained at approximately 7.4.

Tumor tissues were surgically obtained from 51 patients with primary gastrointestinal carcinoma. For primary culture of these carcinomas, the tumor tissue was soaked for approximately 10 min in an antibiotic solution consisting of DMEM, 10% heat-inactivated FBS, 100 μg/ml sodium piperacillin, 5 μg/ml gentamicin and 5 μg/ml amphotericin B. This soaking procedure was repeated between seven and 10 times using fresh antibiotic solution each time. The soaked tumor tissue was minced with trimming blades into cubes of less than 1 mm^3. Then the tumor fragments were placed in a trypsinizing flask containing 10% DMEM and 0.14% type I collagenase, and were enzymatically digested for 30 min at 37°C with stirring. A single-cell suspension containing tumor cells was superinoculated onto fixed-contact-sensitive plates (F-CSPs) in 10% DMEM and 5 μg/ml gentamicin. The number of viable cells was determined by counting with a hemocytometer after staining with 0.4% Trypan blue dye.

Preparation of F-CSPs

Confluent monolayers were prepared by inoculating 10^4 3T3 cells/plate into multiplates (Corning, no. 25820 MP 24F). The medium was changed at confluence and the plates were incubated for an additional 2 days to ensure complete confluence. The confluent monolayers of contact-sensitive 3T3 cells were then fixed with 3% glutaraldehyde and resulting monolayers were termed F-CSPs.[10] Primary cultured normal cells exhibiting contact sensitivity are not able to proliferate on F-CSPs, whereas neoplastic cells were capable of growing.[10]

Treatment of cells with anticancer agents

Anticancer agents were prepared in phosphate buffer solution (PBS) and stored at −70°C for individual assays. The following drug concentrations for testing were selected on the basis of 1/10 of peak plasma concentrations achieved during clinical administration:[6] SN-38, 6.25 ng/ml; ADM, 0.04 μg/ml; MMC, 0.1 μg/ml; cisplatin, 0.3 μg/ml; and 5-FU, 0.5 μg/ml.

Cell lines or primary cultured cancer cells were seeded on F-CSPs at a density of approximately 10^3 or 10^4 cells, respectively, and the cultures were incubated for 48 h without a medium change. Then the cultures were treated with each of the anticancer agents except 5-FU for 2 h, and with 5-FU for 4 days.[11] Control cultures were incubated at 37°C for the same period. After the treatment, the cells were washed three times with fresh medium.

The cell lines and the primary tumor cells were cultured on F-CSPs in 10% DMEM in a humidified atmosphere of 5% CO_2/95% air. The pH of the culture medium was maintained at approximately 7.4.

Quantitative measurements of cancer cell growth on F-CSPs

The growth of cell lines and primary cancer cells on F-CSPs was evaluated by measuring [^{3}H]dThd incorporation into DNA and by counting the number of colonies.

For the dThd incorporation assay, 1 μCi/ml [^{3}H]dThd was added to each culture plate and incubation was continued for 3 h at 36°C. The plate was subsequently washed five times with PBS and then treated with 10% TCA. After centrifugation at 3000 *g* for 20 min, the pellets were dissolved in 0.3 N KOH and kept at 37°C for 20 h. The level of radioactivity was determined by using an ACS-II scintillator. Cell survival was expressed as the percentage of the radioisotope incorporated into the treated culture relative to that into the untreated cultures using the following formula: cell survival (%) $= T/C \times 100$, where T is the c.p.m. of treated cells and C is the c.p.m. of untreated cells.

For the clonogenic assay, 200 cells of the KP-1N and MKN-45 lines were superinoculated onto F-CSPs prepared in 60-mm culture dishes (Corning, no. 25010). After 7 days of culture, the number of colonies was counted using a stereoscopic microscope and the clonogenic efficiency was expressed as the percentage of colonies in the treated plates versus the untreated control plates.

Statistical analysis

Student's *t*-test was used to determine the significance of inter-group differences in cell survival. The χ^2 test was used to determine significant differences in response rates of cases between two groups.

Results

Evaluation of cytotoxicity of CPT-11 (SN-38) for KP-1N and MKN-45 cell lines

Figure 1 shows the percentage survival as determined by both the colony forming assay and [^{3}H]dThd incorporation when KP-1N and MKN-45 cell lines were treated with CPT-11 (SN-38). The percent survival of KP-1N and MKN-45 cells shown by the colony forming assay was correlated with that shown by [^{3}H]dThd incorporation. Therefore, the survival determined by [^{3}H]dThd incorporation appeared to reflect the cytotoxicity for both KP-1N and MKN-45 cells. When the percent survival of these two cell lines shown by the colony forming assay was 50%, that shown by [^{3}H]dThd incorporation was approximately 75%. Thus, if the percent survival of primary cultured cells shown by [^{3}H]dThd incorporation is under 75%, the clonogeneic efficiency (cytotoxicity) might be under 50%. Accordingly, the cut-off survival level of primary cancer cells for the [^{3}H]dThd assay was set at 75%, i.e. sensitive cells (high response cases) showed 75% or lower survival and resistant cells (low response cases) showed more than 75% survival.

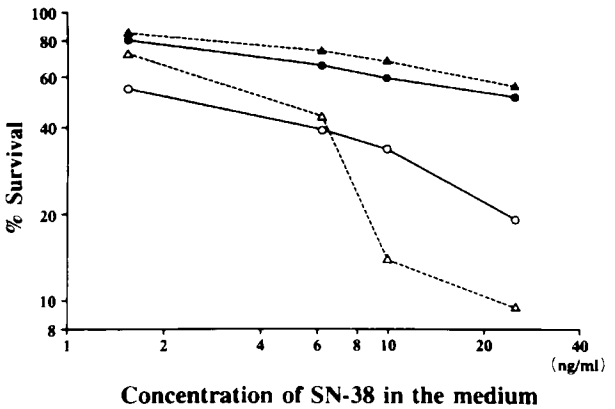

Figure 1. Evaluation of the cytotoxicity of CPT-11 (SN-38) for two cell lines. The persent survival of KP-1N cells is shown by [^{3}H]dThd incorporation (●) and by clonogenic efficiency (○). The percent survival of MKN-45 cells is shown by [^{3}H]dThd incorporation (▲) and by clonogenic efficiency (△).

Growth inhibitory effect of CPT-11 (SN-38) for primary cultures of gastrointestinal carcinomas

The growth inhibitory effect of SN-38 for primary cultured gastrointestinal carcinomas was examined and compared with the effect of various other anticancer agents (Table 1). The averages of percent survivals of gastrointestinal carcinomas (stomach, colon, hepatocellular and pancreas cancer) were 77.8% for SN-38, 82.0% for cisplatin, 75.5% for MMC, 75.4% for ADM and 87.9% for 5-FU. There were no statistical differences among these five anticancer drugs.

The average percent survivals of stomach, colon, hepatocellular and pancreas cancers against SN-38 were 82.6, 71.6, 77.5 and 79.4%, respectively. These averages for SN-38 were not different from those of any other anticancer agents.

Rate of cases having a high response (survival rate 75% or less) to CPT-11 (SN-38)

Rates of cases showing a high response (percent survival 75% or less) to SN-38, cisplatin ADM and 5-FU were 21.4, 16.4, 21.4 and 10.4%, respectively (Table 2). Rates of cases with a high response to SN-38 were 9.10, 31.3, 25.0 and 20.0% in stomach, colon, hepatocellular and pancreas cancers, respectively, and they were not significantly lower than those to other drugs in each of the gastrointestinal carcinoma.

Rate of cases showing a high response to CPT-11 (SN-38) but a low response to other anticancer drugs in comparison with that of cases showing low response to SN-38 but high response to other anticancer drugs.

As shown in Table 3, the rates of cases with a high response (percent survival 75% or less) to CPT-11 (SN-38) but a low response (percent survival above 75%) to cisplatin, MMC, ADM or 5-FU were 14.6, 19.4, 15.6 and 27.0%, respectively. In contrast, the rates of cases having a low response to SN-38 but a

Table 1. Growth inhibitory effect of CPT-11 (Sn-38) against gastrointestinal carcinomas

Origin	Case	Average survival (%)				
		SN-38 (6.25 ng/ml)	Cisplatin (0.3 μg/ml)	MMC (0.1 μg/ml)	ADM (0.04 μg/ml)	5-FU (0.2 μg/ml)
Stomach	(22)	82.6	82.2	81.9	76.9	92.9
Colon	(16)	71.6	82.3	67.7	72.8	83.6
Liver	(8)	79.4	84.2	68.3	73.2	82.9
Pancreas	(5)	77.5	79.3	84.0	78.8	92.1
Mean ± SD	(51)	78.1 ± 21.7	82.2 ± 17.7	76.8 ± 24.6	75.1 ± 23.4	87.6 ± 21.1

Table 2. Rates of cases having a high response to CPT-11 (SN-38) against gastrointestinal carcinomas

Origin	Rates of cases having percent survival ≤ 75%				
	SN-38 (6.25 ng/ml)	Cisplatin (0.3 μg/ml)	MMC (0.1 μg/ml)	ADM (0.04 μg/ml)	5-FU (0.2 μg/ml)
Stomach	27.2 (6/22)	29.4 (5/17)	17.6 (3/17)	23.1 (3/13)	16.7 (2/12)
Colon	43.8 (7/16)	30.8 (4/13)	37.5 (3/8)	44.4 (4/9)	33.3 (4/12)
Liver	37.5 (3/8)	33.3 (2/6)	66.7 (4/6)	57.1 (4/7)	12.5 (1/8)
Pancreas	40.0 (2/5)	20.0 (1/5)	20.0 (1/5)	33.3 (1/3)	20.0 (1/5)
	35.3 (18/51)	29.3 (12/41)	30.6 (11/36)	37.5 (12/32)	21.6 (8/37)

Table 3. Cases with high response to CPT-11 (SN-38) but low response to other anticancer drugs in comparison with those with low response to SN-38 but high response to other drugs

Origin	Rates of cases (%)			
	Cisplatin (0.3 μg/ml)	MMC (0.1 μg/ml)	ADM (0.04 μg/ml)	5-FU (0.2 μg/ml)
Cases having percent survival for SN-38 ≤ 75% but for the other anticancer drugs > 75%				
stomach	17.6 (3/17)	23.5 (5/17)[a]	15.4 (2/13)	16.7 (2/12)
colon	15.4 (2/13)	25.0 (2/8)	22.2 (2/9)	33.3 (4/12)
liver	16.7 (1/6)	16.7 (1/6)	0 (0.7)	25.0 (2/8)
pancreas	0 (0/5)	0 (0/5)	33.3 (1/3)	40.0 (2/5)
	14.6 (6/41)	19.4 (7/36)[b]	15.6 (5/32)	27.0 (10/37)
Cases having percent survival for the other anticancer drug ≤ 75%, but for SN-38 > 75%				
stomach	11.8 (2/17)	5.9 (1/17)[c]	0 (0/13)	16.7 (2/12)
colon	7.7 (1/13)	0 (0/8)	11.1 (1/9)	16.7 (2/12)
liver	0 (0/6)	0 (0/6)	0 (0/7)	12.5 (1/8)
pancreas	0 (0/5)	0 (0/5)	33.3 (1/3)	0 (0/5)
	7.3 (3/41)	2.8 (1/36)[d]	6.3 (2/32)	13.5 (5/37)

[a–c] $p = 0.08$; [b–d] $p = 0.03$.

high response to cisplatin, MMC, ADM or 5-FU were 7.3, 2.8, 9.4 and 13.5%, respectively. The rates of cases with a high response to SN-38 but a low response to the other drugs were higher than those of cases having a low response to SN-38 but a high response to the other drugs. Especially, for MMC, the former rate (19.4%) was statistically significantly higher than the latter rate (2.8%) ($p = 0.03$). In colon cancer patients, there were three cases showing a low response (percent survival: 59.4, 69.2 and 39.9%) for treatment with SN-38 but not with any other drugs. For liver cancer, the cases having a high response to cisplatin and MMC showed a low response to SN-38.

Growth inhibitory effect of CPT-11 (SN-38) on cancer cells obtained from primary or metastatic lesions

As shown in Table 4, the growth inhibitory effect of CPT-11 (SN-38) against metastatic lesions of gastrointestinal carcinomas was compared with that against primary lesions. There was no statistically significant difference of percent survival of cancer cells obtained from between primary and metastatic lesions in total cases. Similar results were gained in stomach and colon cancer cases. However, in pancreatic cancer, the mean percent survival of metastatic lesions was significantly lower than that of primary lesions ($p = 0.03$). The average percent survival of primary lesions in colon cancer was significantly lower than that in stomach cancer ($p = 0.08$).

Table 4. Growth inhibitory effect of CPT-11 (SN-38) against gastrointestinal carcinoma in primary or metastatic sites

Origin	Site	Case	Percent survival (%)
Stomach	primary	14	85.6±15.7[a]
	metastasis	8	77.4±30.3
Colon	primary	6	64.4±33.3[b]
	metastasis	10	75.8±16.4
Liver	primary	8	79.4±17.6
Pancreas	primary	2	98.6±4.81[c]
	metastasis	3	63.5±12.7[d]
Total	primary	30	80.6±21.6
	metastasis	21	74.7±21.9

[a–b] $p = 0.08$; [c–d] $p = 0.03$.

Discussion

CPT-11 is a semisynthetic derivative of camptothecin and SN-38 is the major metabolite of CPT-11; both agents inhibit topoisomerase I activity and thus inhibit nucleic acid synthesis. CPT-11 has shown antitumor activity against cervical, ovarian and lung cancers in clinical studies,[12,13] and also inhibited the experimental and spontaneous metastasis of highly metastatic tumors.[14] Furthermore, it has been shown to be effective against multidrug-resistant human tumor cell lines.[15] It is generally accepted that the inhibition of topoisomerase I by CPT-11 is mainly attributable to its metabolite SN-38.[3] Therefore, it seemed to be important that the cytotoxicity of SN-38 was evaluated when the inhibitory effect of CPT-11 on neoplastic cells was examined. We previously reported that SN-38 had a cytotoxic effect against cultured cells originating from gastrointestinal carcinomas, even when the other anticancer agents achieved no response, and from recurrent carcinomas after treatment with various agents.[16]

In the present study, we used the F-CSP assay[6] to assess the cytotoxic effect of SN-38 on primary and metastatic gastrointestinal carcinomas separated from stomach, colon, liver and pancreas. In addition, we examined how many cases showing a high response to CPT-11 (SN-38) but a low response to cisplatin, MMC, ADM and 5-FU there were. When CPT-11 is administered i.v. at a dose of 100–250 mg/m^2, the peak plasma concentration of SN-38 was 33–72 ng/ml.[17,18] We examined the sensitivity of tumors to SN-38 by consideration of these concentrations[19] and made a comparison with the response to other anticancer drugs.[16]

With exposure of 6.25 ng/ml SN-38, there was no significant difference in the average percent survivals among the origins of the cultured cells of gastrointestinal carcinoma. The rate of cases with a high response (percent survival 75% or less) of these carcinoma cells to SN-38 was not different from that to the other anticancer agents. However, the rates of cases having a high response to SN-38 but a low response (percent survival above 75%) to the other anticancer drugs were higher than those of the opposite cases (a low response to SN-38 but a high response to other anticancer drugs). In addition, cell growth of three cases (18.8%, 3/16) with colon cancer was inhibited by SN-38 but not inhibited by the other anticancer drugs. The growth inhibitory effect of SN-38 for primary lesions of colon cancer was almost equal to that of metastatic lesions, and was significantly superior to that for primary lesions of stomach cancer. In an early

phase II trial, the response of CPT-11 for metastatic colon cancer was reported in 27% of 63 patients.[20] This response rate is regarded as a high level for colon cancer in a single modality treatment. CPT-11 will be expected to be one of the major anticancer drugs for colon cancer.

In eight hepatocellular carcinoma cases, SN-38 showed a high response of 37.5%, and the rates of cases having a high response to SN-38 but a low response to cisplatin, MMC, ADM and 5-FU were 16.7, 16.7, 0 and 25%, respectively. However, only one case showed a high response to 5-FU but a low response to SN-38. This shows that it is possible that CPT-11 might be effective on hepatocellular carcinoma cases without a response to cisplatin, ADM and 5-FU. Phase II studies of hepatocellular carcinomas have not been done yet, but should be tried in the near future.

SN-38 showed a high response of 40% in five pancreatic cancer cases, and only one case with pancreatic cancer had a low response to SN-38 but a high response to ADM and other anticancer drugs. The mean percent survival of metastatic lesions (63.5%) was significantly lower than that of primary lesions. CPT-11 inhibited spontaneous and experimental metastases of several highly metastatic variants of murime adenocarcinoma.[14] Combined treatment with CPT-11 and some other anti-cancer drug may be effective on pancreatic cancer because none of the other tested drugs exhibited any effect of a single modality treatment against pancreatic cancer.

This study suggests that CPT-11 has a clinically high response to colon and hepatocelluar cancers. Therefore, we recommend further clinical studies on CPT-11 for these cancers.

References

1. Tsuro T, Matsuzaki T, Matsushita M, *et al.* Antitumor effect of CPT-11, a new derivative of camptothecin, against pleotropic drug-resistant tumors *in vitro* and *in vivo*. *Cancer Chemother Pharmacol* 1988; **21**: 71–4.
2. Kaneda N, Nagata H, Furuta T. Metabolism and pharmacokinetics of the camptothecin analogue CPT-11 in the mouse. *Cancer Res* 1990; **50**: 1715–20.
3. Kawato Y, Aonuma M, Hirota Y, *et al.* Intracellular roles of SN-38, a metabolite of the camptothecin derivative CPT-11, in the antitumor effect of CPT-11. *Cancer Res* 1991; **51**: 4187–91.
4. Kawato Y, Furuta T, Aonuma N. Antitumor activity of a camptothecin derivative, CPT-11, against human tumor xenografts in nude mice. *Cancer Chemother Pharmacol* 1991; **28**: 192–8.
5. Negoro S, Fukuoka M, Matsuda N. Phase I study of weekly intravenous infusions of CPT-11, a new derivative of camptothecin, in the treatment of advanced non-small-cell lung cancer. *J Natl Cancer Inst* 1991; **83**: 1164–8.
6. Matsuoka H, Ueo H, Yano K, *et al.* Estradiol sensitivity test using contact-sensitive plates of confluent Balb c 3T3 cell monolayers. *Cancer Res* 1990; **50**: 2113–8.
7. Kakunaga T. A quantitative system for assay of malignant transformation by chemical carcinogens using clone derived from Balb/c 3T3. *Int J Cancer* 1973; **12**: 463–73.
8. Matsuoka H, Mori M, Ueo H, *et al.* Characterization of human esophageal carcinoma cell line established on confluent monolayer, and advantage of confluent monolayer surface structure for attachment and growth. *Pathobiology* 1991; **59**: 76–84.
9. Halto H. Establishment of cultured cell lines of human stomach cancer origin and their morphological characteristics. *Niigata Med* 1977; **91**: 737–52.
10. Matsuoka H, Furusawa M, Tomoda H, *et al.* Use of fixed contact-sensitive monolayers fror identification and separation of normal from neoplastic cells. *J Tiss Cult Methods* 1993; **15**: 185–9.
11. Matsuoka H, Furusawa M, Tomoda H, *et al.* Increased cytotoxicity of 5'-deoxy-5-fluorouridine by prolonged culture with folinic acid. *Anticancer Res* 1993; **13**; 2201–6.
12. Takeuchi S, Tsuchihashi K, Fujimoto S, *et al.* A late phase II study of CPT-11 in uterine cervical cancer and ovarian cancer. *Jpn J Cancer Chemother* 1991; **18**: 1681–9.
13. Fukuoka M, Niitani H, Suzuki A, *et al.* A phase II study of CPT-11, a new derivative of camptothecin, for previously untreated non-small-cell lung cancer. *J Clin Oncol* 1992; **10**: 16–20.
14. Matsuzaki T, Yokokura T, Mutai M, *et al.* Inhibition of spontaneous and experimental metastasis by a new derivative of camptothecin, CPT-11, in mice. *Cancer Chemother Pharmacol* 1988; **21**: 308–12.
15. Niimi S, Nakagawa K, Sugimoto Y, *et al.* Mechanism of cross-resistance to a camptothecin analogue (CPT-11) in a human ovarian cancer cell line selected by cisplatin. *Cancer Res* 1992; **52**: 328–33.
16. Matsuoka H, Furusawa M, Tomoda H, *et al.* Cytotoxicity of CPT-11 and SN-38 for gastrointestinal and recurrent carcinomas cultured on contact-sensitive plates. *Anticancer Res* 1994; **14**: 405–410.
17. Taguchi T, Wakui A, Hasegawa K, *et al.* Phase I clinical study of CPT-11. *Jpn J Cancer Chemother* 1990; **17**: 115–120.
18. Negoro S, Fukuoka M, Masuda N, *et al.* Phase I study of weekly intravenous infusions of CPT-11, a new derivate of camptothecin, in the treatment of advanced non-small-cell lung cancer. *J Natl Cancer Inst* 1991; **83**: 1164–8.
19. Von Hoff DD, Casper J, Bradley E, *et al.* Association between human tumor colony-forming assay results and response of an individual patient's tumor to chemotherapy. *Am J Med* 1981; **70**: 1027–32.
20. Shimada Y, Yoshino M, Wakui A, *et al.* Phase II study of CPT-11, a new camptothecin derivative, in metastatic colorectal cancer. *J Clin Oncol* 1993; **11**: 909–13.

(Received 16 February 1995; accepted 23 February 1995)