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Activity of CPT-11 (irinotecan hydrochloride), a topoisomerase I inhibitor, against human tumor colony-forming units

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CPT-11 (irinotecan hydrochloride, 7-ethyl-10-[4-(piperidino)-1-piperidino] carbonyloxy-camptothecin) is a semisynthetic camptothecin derivative developed in Japan. The inhibitory activity of CPT-11 against human tumor colony-forming units from freshly explanted human tumors was explored using a soft agar cloning system. Final CPT-11 concentrations of 0.3–3.0 μg/ml were used for a 1 h exposure. At a concentration of 3.0 μg/ml antitumor activity was seen against colorectal, ovarian, non-small-cell lung, breast cancer and mesothelioma colony-forming units. CPT-11 should have activity against a broad spectrum of tumors in patients.

Key words: Cloning system, CPT-11, human tumor colony-forming units, irinotecan.

Introduction

CPT-11 (irinotecan hydrochloride, 7-ethyl-10-[4-piperidino)-1-piperidino] carbonyloxy-camptothecin) is a new analog of the plant alkaloid, camptothecin,[1] which is an extract from *Camptotheca acuminata*. In the early 1970s, captothecin was studied for efficacy in the US.[2,3] However, clinical development was abandoned due to unexpectedly severe clinical toxicities including hemorrhagic cystitis, neutropenia and gastrointestinal toxicities. In 1983, CPT-11 was developed by Kunimoto and colleagues.[4] It demonstrated very significant antitumor activity in a variety of preclinical systems. CPT-11 exerts its antitumor activity through the inhibition of topoisomerase I, which is an essential enzyme for DNA replication. This mechanism is a novel target for cancer chemotherapy. We have reported the inhibitory activity of topotecan, another topoisomerase I inhibitor, against human tumor colony-forming units (HTCFUs) in a previous report.[5] Topotecan showed *in vitro* activity against colorectal, breast, lung, ovarian and renal cell cancer HTCFUs.

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In the present investigation we have examined the *in vitro* activity of CPT-11 against a variety of HTCFUs using a soft agar cloning system. The study was performed to select specific tumor types in which CPT-11 should have phase II clinical trials as well as determine plasma concentrations which need to be achieved to expect responses in patients.

Materials and methods

Compounds

CPT-11 was kindly supplied by Yakult Honsha Co. (Tokyo, Japan). Final concentrations (dissolved in distilled water) ranged from 0.3 to 3.0 μg/ml.

Human tumor cloning system

After obtaining written informed consent from patients in accordance with federal and institutional guidelines, tumor specimens were collected by standard sterile procedures as a part of routine clinical practice.

Biopsy specimens of solid tumors were placed in McCoy's 5A medium containing 10% newborn calf serum, 10 mM HEPES, 90 U/ml penicillin and 90 μg/ml streptomycin (all from Gibco BRL, Grand Island, NY), for transportation to the laboratory. Preservative-free heparin (10 U/ml; O'Neill, Johns and Feldman, St Louis, MO) was added immediately after collection of fluids to prevent coagulation.

Solid specimens were minced and repeatedly passed through metal meshes with widths of 40 μm (EC Apparatus, St Petersburg, FL) to obtain a single-cell suspension. Effusions were centrifuged at 150 *g* for 5–7 min and passed through 25 gauge needles to obtain a single-cell suspension. Cells

were suspended in McCoy's 5A medium containing 5% horse serum and 10% fetal calf serum (both Hyclone, Logan, UT) and 2 mM sodium pyruvate, 2 mM glutamine, 90 U/ml penicillin, 90 μg/ml streptomycin and 35 μg/ml L-serine (all from Gibco BRL).

The human tumor cloning assay was performed with the two-layer system described by Hamburger and Salmon,[6] with several modifications. Base layers contained 0.5% agar (Difco, Detroit, MI) in the mixture of McCoy's 5A medium mentioned above, 0.6% soy broth (Difco Laboratories) and 100 μg/ml asparagine (Gibco BRL). Cells were plated in a top layer at the density of 5×10^5 per dish in 35 mm Petri dishes (Corning Science Products, Corning, NY) in a mixture of 0.3% agar in Connaught Medical Research Laboratories medium 1066 (Irvine Scientific, Irvine, CA) containing 15% horse serum, 2% fetal calf serum, 5 mg/100 ml vitamin C, 90 U/ml penicillin, 90 μg/ml streptomycin, 0.1 mM non-essential amino acids, 2 mM glutamine (all from Gibco BRL), 2 U/ml insulin (Iletin IR; Eli Lilly, Indianapolis, IN), 2 μg/ml transferrin and 4 ng/ml hydrocortisone (both from Sigma, St Louis, MO). Immediately before cells were plated, HEPES (Gibco BRL; 10 mM final concentration) and sodium pyruvate (2 mM final concentration) were added. Tumor cells were incubated with CPT-11 for 1 h and the cells were washed twice with McCoy's 5A medium and 10% heat-inactivated fetal calf serum, and then plated. All determinations were performed in triplicate. Each experiment included a control with orthosodiumvanadate (0.01 M; Sigma) to assure the presence of a good single-cell suspension (positive control). Plates were incubated at 37°C under 5% CO_2 and 100% humidity. After 14 days, colonies were counted under an inverted microscope.

An experiment was considered evaluable when the distilled water control (without CPT-11) had 20 or more colonies per plate and when the positive control (orthosodium vanadate) showed inhibition of colony formation of at least 70%. A decrease in tumor cell colony formation resulting from exposure to CPT-11 was considered to be a positive response if survival of colonies was 50% or less than that of water controls.

Statistical analyses

Data were expressed as means and standard deviations of triplicate determinations per datapoint. We calculated percent survival by expressing the average number of tumor colony-forming units per Petri dish from CPT-11 treated cells as a percent of the average number of tumor colony-forming units per Petri dish from untreated controls. Concentration versus *in vitro* response data were analyzed utilizing a repeated measures analysis of variance. Comparisons of cross-resistance with other agents were analyzed utilizing McNemar tests.

Results

A total of 220 freshly explanted human tumor specimens were plated in the human tumor cloning assay. Six of these specimens were diagnosed as benign and did not form colonies in controls. These specimens were excluded from further analysis. Of 214 tumor specimens, 97 (45%) specimens were evaluable (Table 1). Tumor types studied are also summarized in Table 1. The major tumor subtypes were colorectal, ovarian, non-small-cell lung, breast cancers and mesothelioma. *In vitro* activity of CPT-11 was examined at three different concentrations. Tumor colony-forming units were inhibited more than 50% of controls, in 8 of 93 (9%) evaluable specimens exposed to CPT-11 at the concentration of 0.3 μg/ml, in 20 of 97 (21%) at 1.5 μg/ml and in 32 of 95 (34%) at 3.0 μg/ml, respectively (Table 2). A concentration-dependent increase in the frequency of significant inhibition (>50%) was seen ($p = 0.0001$) for 1.5 versus 0.3 μg/ml and for 3.0 versus 1.5 μg/ml ($p = 0.001$). *In vitro* sensitive tumor types included colorectal, ovarian, non-small cell lung, breast cancers and mesothelioma,

Table 1. Tumor types studied with CPT-11

Tumor type	No. evaluable	No. attempted	(%)
Colorectal	20	53	(38)
Ovary	23	28	(82)
Lung (non-small-cell)	11	20	(55)
Mesothelioma	11	18	(61)
Breast	9	17	(53)
Melanoma	5	8	(63)
Cervix	2	4	(50)
Pancreas	2	3	(67)
Sarcoma	2	11	(18)
Skin	1	2	(50)
Unknown primary	3	9	(33)
Other[a]	8	41	(20)
Total	97	214	(45)

[a]Other category include 0/2 testis, 1/3 prostate, 0/1 carcinoid, 1/4 corpus uteri, 1/1 small bowel, 1/1 gallbladder, 2/7 kidney, 1/4 liver, 0/5 head and neck, 0/3 small-cell lung, 0/3 lymphoma, 0/2 myeloma and 1/5 stomach.

Table 2. Concentration-dependent inhibition of colony formation by CPT-11 after 1 h exposure

Tumor type	No. of specimens with inhibition[a]/no. of specimens evaluable at CPT-11 concentration (μ/ml)		
	0.3	1.5	3.0
Colorectal	1/20	2/20	5/20
Ovary	2/22	3/23	5/22
Lung (non-small-cell)	1/11	4/11	6/11
Breast	1/8	3/9	5/8
Mesothelioma	1/10	2/11	4/11
Cervix	1/2	1/2	1/2
Melanoma	0/4	1/5	1/5
Pancreas	0/2	1/2	1/2
Sacoma	0/2	1/2	2/2
Skin	0/1	1/1	1/1
Unknown primary	1/3	1/3	1/3
Other[b]	0/8	0/8	0/9
Total (%)	8/93 (9)[c]	20/97 (21)[c,d]	32/95 (34)[d]

[a]Colony survival ≤0.5 × control.
[b]Other category includes two evaluable renal cells, and one each of corpus uteri, small bowel, gallbladder, hepatoma, prostate and stomach.
[c]$p = 0.0001$ for comparison by repeated measures analysis of variance.
[d]$p = 0.001$ for comparison by repeated measures analysis of variance.

with responses observed in 25, 23, 55, 63 and 36%, respectively, of assessable tumor specimens at a CPT-11 concentration of 3.0 μg/ml (Table 2). Although the number of evaluable tumors studied was small, activity was also noted against cervix, melanoma, pancreas, squamous cell skin and sarcoma colony-forming units.

We compared *in vitro* responses to CPT-11 at a concentration of 3.0 μg/ml with those to seven conventional antineoplastic agents (Table 3). In head-to-head comparison, tumor cells were significantly more likely to be sensitive to CPT-11 than to doxorubicin. Thus HTCFUs resistant to doxorubicin are likely not to be cross-resistant to CPT-11. For the other drugs studied, there was not a significant incidence of non-cross-resistance. This may be due to the relatively small sample sizes for each agent.

Discussion

In the 1970s, camptothecin sodium was studied in the US, but unexpectedly severe toxicities such as hemorrhagic cystitis, neutropenia and gastrointestinal toxicity were noted.[2,3] Although some responses were seen, further development was abandoned due to clinical toxicities.[7] New, less-toxic derivatives were necessary for clinical development.

In the late 1980s, some active derivatives (9-aminocamptothecin[7] and topotecan) were tested in preclinical systems. CPT-11 is currently being evaluated in ongoing clinical trials. This compound has a unique structure, with a piperidio- side chain at the 10th position of camptothecin. This modification gives improved water solubility and fewer toxicities in preclinical systems. CPT-11 has shown broad and significant antitumor activity against a variety of preclinical tumor types.[8,9] The mechanism of action of CPT-11 is the inhibition of topoisomerase I activity.

We have studied the activity of CPT-11 as a 1 h exposure against HTCFUs from freshly explanted human tumors. CPT-11 inhibited HTCFUs in a concentration-dependent fashion. Significant *in vitro* activity is seen when *in vitro* concentrations of CPT-11 were ≥1.5 μg/ml (see Table 2). Thus, if one could achieve similar concentrations in patients, CPT-11 should have clinical antitumor activity.

Some groups have already reported pharmacokinetic parameters for CPT-11. In a phase I study by Taguchi and colleagues,[10] CPT-11 was administered with 30 min infusion every 3 weeks with doses escalated from 50 to 350 mg/m^2. Maximum peak plasma concentrations (C_{max}) of CPT-11 ranged from 0.72 to 7.58 μg/ml.[10] Sasaki *et al.*[11] reported a C_{max} of 1.35 μg/ml (range from 0.74 to 2.31 μg/ml) with a dose of 100 mg/m^2 as a 90 min infusion. Negoro *et al.*[12] reported a C_{max} of 1.58 μg/ml at a dose of 125 mg/m^2 and a C_{max} of 2.97 μg/ml at a dose of 150 mg/m^2 as a 90 min infusion. Similarly, our group reported a C_{max} of 1.41 μg/ml at 100 mg/m^2 as a 90 min infusion.[13] The Johns Hopkins' group reported a C_{max} of 3.39 μg/ml at 290 mg/m^2 given over 90 min.[14] Finally, the French group administered CPT-11 at a dose of 100 mg/m^2 over 30 min and obtained a peak plasma level of 2.57 μg/ml.[15] In summary, the pharmacokinetic data from phase I studies indicated that peak plasma levels ranged from 0.72 to 7.58 μg/ml. Thus, it appears the concentrations we tested *in vitro* can be achieved in patients.

At the concentration of 3.0 μg/ml, HTCFUs sensitive *in vitro* to CPT-11 included breast (5/8), non-small-cell lung (6/11), mesothelioma (4/11), ovarian (5/22) and colorectal cancer (5/20). Other types responding *in vitro* (with only a small sample size tested) included sarcomas, cervical cancer, melanoma, pancreatic cancer and squamous cell skin cancer. In early Japanese clinical trials, antitumor

Table 3. Comparison of the antitumor activity in tumor specimens with 1 h exposure to CPT-11 or conventional antineoplastic agents

Drug	CPT-11(3.0 μg/ml)		Total Specimens	p value
	no. sensitive	no. resistant		
Doxorubicin (0.4 μg/ml)				
no. sensitive[a]	6	2		
no. resistant	10	22		
			40	0.043
Cisplatin (0.2 μg/ml)				
no. sensitive	6	4		
no. resistant	11	16		
			37	0.121
Mitomycin-C (0.1 μg/ml)				
no. sensitive	2	0		
no. resistant	5	17		
			24	0.074
Vinblastine (0.05 μg/ml)				
no sensitive	7	3		
no. resistant	3	11		
			24	1.0
5-Fluorouracil (6.0 μg/ml)				
no. sensitive	3	3		
no resistant	3	12		
			21	+.0
4-Hydroperoxy cyclophosphamide (3.0 μg/ml)				
no. sensitive	3	2		
no. resistant	4	7		
			16	0.683
Etoposide (3.0 μg/ml)				
no. sensitive	1	1		
no. resistant	4	6		
			12	0.371

[a]Colony formation ≤0.5 × control.

activity for CPT-11 has already been reported for patients with non-small-cell lung,[16] small-cell lung,[17] ovarian,[18] colorectal[19] and breast cancers. Additional phase II clinical trials are ongoing. Our *in vitro* studies indicate these phase II trials should demonstrate good clinical activity.

CPT-11 demonstrated incomplete cross-resistance *in vitro* with doxorubicin. These data suggest that a combination of CPT-11 with doxorubicin would be worth pursuing. Masuda *et al.*[20] have reported a high response rate with the combination of CPT-11 with cisplatin for patients with non-small-cell lung cancer. We could not demonstrate non-cross-resistance between CPT-11 and cisplatin in our *in vitro* system ($p = 0.121$, see Table 3). This could be due to the small sample size.

Conclusion

In summary, we have demonstrated that CPT-11 (at clinically achievable concentrations) is cytotoxic against *in vitro* tumor colony-forming units from human tumor specimens. Good *in vitro* activity was demonstrated against non-small-cell lung, ovarian, colorectal and breast cancers as well as mesothelioma. Incomplete cross-resistance was observed with doxorubicin. Further clinical development of CPT-11 is warranted. Combination chemotherapy of CPT-11 plus doxorubicin should be investigated.

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