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Antitumor activity of a camptothecin derivative, CPT-11, against human tumor xenografts in nude mice

Yasuyoshi Kawato[1], Tomio Furuta[2], Masashi Aonuma[1], Megumi Yasuoka[1], Teruo Yokokura[2], and Kensuke Matsumoto[1]

[1] Exploratory Research Laboratories, Daiichi Pharmaceutical Co., Ltd, Kita-kasai, Edogawa-ku, Tokyo 134, Japan
[2] Yakult Central Institute for Microbiological Research, Yaho, Kunitachi, Tokyo 186, Japan

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Summary. The antitumor effects of the camptothecin (CPT) derivative CPT-11, 7-ethyl-10-[4-(1-piperidino)-1-piperidino]-carbonyloxycamptothecin, were tested on human tumor xenografts in nude mice. CPT-11 showed antitumor activity higher than that of Adriamycin, 5-fluorouracil, or futraful, with little or no reduction of body weight being observed in the mice. The growth of colon adenocarcinoma Co-4 was significantly inhibited after a single i.v. injection of CPT-11 at 25, 50, or 100 mg/kg. The single i.v. injection was also significantly effective against mammary carcinoma MX-1 and gastric adenocarcinoma St-15. All of the mice bearing MX-1 tumors were cured by the administration of CPT-11 every 4 days for a total of three treatments at a total dose of 200 mg/kg given i.v. or of 400 mg/kg given p.o. Three i.v. or oral treatments were also effective against Co-4, St-15, gastric adenocarcinoma SC-6, and squamous-cell lung carcinoma QG-56. To achieve the same efficacy attained by i.v. injection, however, oral doses 2–4 times higher than the i.v. doses were required. When the total dose was fixed at 100 mg/kg, a triple i.v. injection was most effective, followed by a single i.v. injection and, finally daily p.o. administration for 10 days. Although SN-38 (7-ethyl-10-hydroxycamptothecin), a metabolite of CPT-11, showed much stronger cytotoxic activity in vitro than did CPT-11, its antitumor effects were similar, if not inferior, to those of CPT-11 in vivo at the same dose level. CPT-11 was converted into SN-38 by human tumors, but the sensitivity of these tumors to CPT-11 in vivo was independent of their ability to produce SN-38. These results suggest that CPT-11 may be clinically effective, depending on the schedule of administration, but that its effectiveness is not related to the ability of the tumor to produce SN-38.

Offprint requests to: Y. Kawato, Exploratory Research Laboratories, Daiichi Pharmaceutical Co., Ltd., 16–13, Kita-kasai 1-Chome, Edogawa-ku, Tokyo 134, Japan

Introduction

Camptothecin (CPT), a plant antitumor alkaloid isolated from *Camptotheca acuminata* [23], exhibits antitumor activity against several experimental tumors [6]. In clinical studies, however, it has shown a low antitumor effect and high toxicity [2, 8, 17, 19]. It was recently demonstrated that CPT inhibits type I DNA topoisomerase [9, 10], although many other anticancer drugs affect the activity of type II DNA topoisomerase [11, 20]. In some tumor cells, these enzymes that are essential for mammalian cell growth are present at high levels [1, 7]. Therefore, both enzymes are considered to be new targets in cancer chemotherapy, and much current research on antitumor agents, including CPT analogues, is directed toward them.

7-Ethyl-10-[4-(1-piperidino)-1-piperidino]-carbonyloxycamptothecin (CPT-11) was semisynthesized as a highly active, lowly toxic, and water-soluble derivative of CPT. CPT-11 showed strong antitumor activity against various experimental murine tumors [14] and rat Walker 256 carcinoma [5] on i.p., i.v., or p.o. administration. It was also effective in models of metastatic murine tumors [16] and against pleiotropic drug-resistant tumors [22]. Moreover, the result of a subrenal capsule assay indicated that CPT-11 was as effective against human tumors as other cancer chemotherapeutics such as Adriamycin (ADM) and 5-fluorouracil (5-FU) [24]. In addition to these observations, it was reported that SN-38 (7-ethyl-10-hydroxycamptothecin), which showed less activity against murine tumors in vivo than did CPT-11 [14], was produced from CPT-11 in mouse serum and tissue homogenate and exhibited remarkably high cytotoxicity in vitro [13]. The maintenance of plasma SN-38 concentrations after CPT-11 administration occurred at higher levels than that after SN-38 administration and might be necessary for the antitumor effect of CPT-11 [13]. These results show the need for further nonclinical studies of the activity of CPT-11 and SN-38 against human tumors in vivo and for a test of the ability of human tumors to produce SN-38.

In the present report, we demonstrate that CPT-11 exhibits high antitumor activity against human tumor xeno-

grafts in nude mice on either i.v. or p.o. administration. Furthermore, we indicate that the effects of CPT-11 on human tumors in vivo are equal, if not superior, to those of SN-38 and that they are independent of the ability of the tumors to produce SN-38 from CPT-11.

Materials and methods

Test compounds. CPT-11 and SN-38 (Fig. 1) were semisynthesized and supplied by Yakult Honsha Co. Ltd. (Tokyo, Japan). ADM, mitomycin C (MMC), and 5-FU were purchased from Kyowa Hakko Kogyo Co., Ltd. (Tokyo, Japan). Cisplatin (CDDP) and futraful (FT) were obtained from Nippon Kayaku Co., Ltd., and Taiho Pharmaceutical Co., Ltd. (Tokyo, Japan), respectively.

Animals. Male athymic nude mice (BALB/c-nu/nu) aged 5 or 6 weeks were purchased from Shizuoka Laboratory Animal Center (Hamamatsu, Japan). They were housed in an exclusive experimental room and were given sterilized food and water ad libitum.

Tumors. Poorly differentiated mammary carcinoma MX-1, poorly differentiated gastric adenocarcinoma SC-6, and squamous-cell lung carcinoma QG-56 were supplied by the Cancer Chemotherapy Center, Japanese Foundation for Cancer Research (Tokyo, Japan). Moderately differentiated gastric adenocarcinoma St-15 and poorly differentiated colon adenocarcinoma Co-4 were generously donated by Dr. T. Kubota, School of Medicine, Keio University (Tokyo, Japan). Well-differentiated gastric adenocarcinoma MKN-28 and poorly differentiated gastric adenocarcinoma MKN-45 were graciously provided by Dr. A. Hoshi, National Cancer Center (Tokyo, Japan). These tumor cells were maintained by sequential s.c. transplantations into nude mice.

Evaluation of antitumor activity. Tumor masses maintained in nude mice were excised, cut into fragments (ca. 3-mm cubes), and transplanted s.c. into nude mice. When the estimated tumor volume (TV) in the mice had grown to between 100 and 300 mm^3, the animals were divided into experimental groups of 5 or 6/cage and were treated i.v. or p.o. with a test compound either once (qd × 1) or every 4 days for a total of three doses (q4d × 3). The TV was calculated according to the following formula:

$$TV = L\ (mm) \times W^2\ (mm^2)/2,$$

where *L* and *W* represent the length and the width of the tumor mass, respectively. After the first administration on day 0, the TV and body weight of the mice were measured two or three times a week for 2 or 4 weeks. Then, the tumor masses were excised and weighed. The inhibition rate of tumor growth on the basis of TV (IR) was calculated according to the following formula:

$$IR = (1 - RV_t/RV_c) \times 100\ (\%),$$

where RV_t represents the mean ratio of TV on day *n* to that on day 0 of a treated group and RV_c indicates that of the control group. The largest value for IR was designated as IR_{max}, which indicates the greatest effect of each test compound. The inhibition rate of tumor growth on the basis of tumor weight (IR_{tw}) was calculated according to the following formula:

$$IR_{tw} = (1 - TW_t/TW_c) \times 100\ (\%),$$

where TW_t indicates the mean tumor weight of a treated group and TW_c represents that of the control group. IR_{tw} was statistically analyzed using the Williams-Wilcoxon test (a nonparametric test). The antitumor activity of each test compound was evaluated from IR_{tw} and IR_{max}. The rate of body weight reduction (ΔBW) was calculated according to the following formula:

$$\Delta BW = (1 - BW_n / BW_0) \times 100\ (\%),$$

where BW_n and BW_o represent the mean body weights of mice on day *n* and on day 0, respectively. The maximal value for ΔBW was designated as ΔBW_{max}, from which the toxicity of each test compound to mice was evaluated.

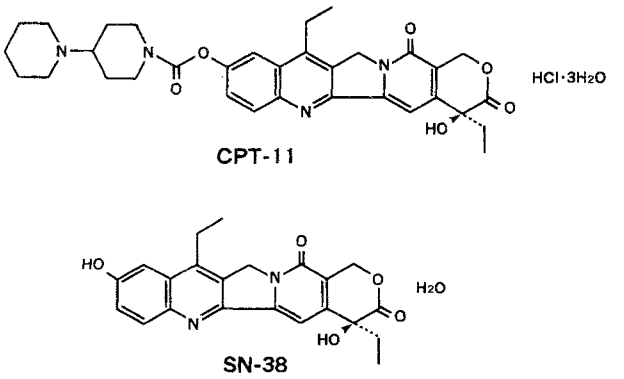

Fig. 1. Molecular structures of CPT-11 and SN-38

Evaluation of cytotoxic activity. In vitro cytotoxic activity was measured by MTT assay essentially as described by Mosmann [18]. Briefly, QG-56 cells were cultured with each test compound in a total of 200 µl RPMI 1640 medium (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) supplemented with 10% fetal bovine serum (HyClone Laboratories Inc., Utah, USA) at 37°C for 4 days in a humidified atmosphere consisting of 5% CO_2 in air. Then, 20 µl tetrazolium dye (MTT; Sigma Chemical Co., St. Louis, Mo., USA) was added to the medium at 5 mg/ml, and the medium was incubated for an additional 4 h. After centrifugation and the removal of 190 µl supernatant, 150 µl dimethylsulfoxide (DMSO) was added and formazan absorbance, which parallels the rate of survival, was measured with a spectrophotometer (Biomek 1000; Beckman Instruments, Inc., California, USA) at test and reference wavelengths of 540 and 660 nm, respectively. The concentration of each test compound required for 50% inhibition of cell growth (IC_{50}) was calculated from the dose-response curve.

Measurement of SN-38 produced from CPT-11 in tumor homogenates. Tumor masses (Co-4, MX-1, SC-6, QG-56) were excised from nude mice at 2–4 weeks after the transplantation. Then, the masses were homogenized in 2 vol. ice-cold 150 mM KCl using a Polytron homogenizer (Model PT10SK; Kinematica, Switzerland) and then centrifuged at 1,500 *g* for 10 min at 4°C. The supernatant was centrifuged at 5,000 *g* for 10 min at 4°C and was adjusted to 14.3 mg protein/ml with 150 mM KCl and then used as a tumor homogenate. A 50-µl sample of 0.3 M TRIS-HCl (pH 7.4) was added to 350 µl tumor homogenate, and the reaction to convert CPT-11 into SN-38 was started by the addition of 100 µl 500 µM CPT-11.

After 2, 5, 10, or 30 min incubation of the reaction mixture (100 µM CPT-11, 10 mg protein/ml) at 37°C, the reaction was terminated by the addition of 1 ml stop solution (90% methanol and 0.04 *N* HCl) under stirring on ice. (For time 0, stop solution was added prior to CPT-11). The mixture was centrifuged at 1,500 *g* for 10 min at 4°C, and the supernatant was filtered through a Shodex DT ED-13 filter (Showa Denko, K.K., Tokyo, Japan). SN-38 in the filtrate was isolated by high-performance liquid chromatography (Senshu Pak ODS-1251-N column; Senshu Scientific Co., Ltd., Tokyo, Japan) with an eluting solution composed of CH_3CN/H_2O (1:2, v/v) and was quantified by fluorospectrometry at an excitation wavelength of 380 nm and an emission wavelength of 556 nm (Fluorescence Spectrophotometer, Model F1000; Hitachi Ltd., Tokyo, Japan).

Results

Effects of a single i.v. dose of CPT-11 on human tumor xenografts

Table 1 summarizes the effects of a single i.v. injection of CPT-11 and of other anticancer agents. At a dose of 100 or

Table 1. Comparison of the antitumor activity of CPT-11 vs other anticancer agents after a single i. v. injection

Tumor	Agent	Total dose (mg/kg)	IR_{max}[a] (%)	IR_{tw}[b] (%)	ΔBW_{max}[c] (%)	Cured mice/ total[d]
Co-4	CPT-11	25	68.9 (11)	62.3**	2.7 (4)	0/6 (28)
		50	75.1 (14)	70.3**	5.6 (2)	0/6
		100	88.6 (18)	75.4**	8.9 (4)	0/6
	ADM	4	24.8 (25)	37	4.1 (4)	0/6
		8	58.2 (28)	60.1*	9.7 (4)	0/6
	CDDP	2.25	59.1 (18)	50	4.1 (2)	0/6
		4.5	72.6 (18)	63 *	5.5 (4)	0/6
		9	92.6 (21)	91.3**	13 (6)	0/6
MX-1	CPT-11	60	71.1 (9)	45.4*	0.8 (5)	0/6 (28)
		100	89.6 (9)	63.1**	1.2 (5)	1/6
	ADM	8	39.6 (16)	42.6	4.9 (5)	0/6
	CDDP	9	58 (12)	31.7	4.9 (5)	0/6
	MMC	6	100 (28)	100 **	8.9 (28)	6/6
St-15	CPT-11	120	64.7 (18)	64.3**,***	−2 (4)	0/5 (31)
	ADM	8	11.2 (18)	18.9	5.2 (4)	0/5
SC-6	CPT-11	100	51.5 (8)	−1.6	3.5 (2)	0/6 (30)
	ADM	8	46.5 (11)	31.9	7.9 (5)	0/6
	CDDP	9	76.9 (11)	21	15.5 (5)	0/6
MKN-28	CPT-11	100	29.7 (19)	31.1	4.2 (2)	0/5 (27)
MKN-45	CPT-11	100	29.8 (8)	17.4	6 (5)	0/6 (15)
QG-56	CPT-11	100	23.9 (22)	−5.2	4.7 (12)	0/6 (28)
	ADM	8	44.9 (19)	19.8	7.1 (4)	0/6

[a] Maximal tumor growth-inhibition rate calculated from the estimated tumor volume; numbers in parentheses indicate the days on which IR_{max} was reached after drug administration on day 0

[b] Tumor growth-inhibition rate calculated from the tumor weight: * $P < 0.05$, ** $P < 0.01$ as compared with untreated controls; *** $P < 0.05$ as compared with ADM at 8 mg/kg

[c] Maximal rate of body weight reduction, numbers in parentheses denote the days of ΔBW_{max}

[d] Numbers in parentheses indicate the days on which the tumor weight and number of mice cured were measured

120 mg/kg, CPT-11 was significantly effective against Co-4, MX-1, and St-15 tumors, yielding IR_{tw} values of 75.4%, 63.1%, and 64.3%, respectively, but was not effective against SC-6, MKN-28, MKN-45, or QG-56 tumors.

CPT-11 significantly inhibited the growth of Co-4 at doses of 25–100 mg/kg and showed activity that was similar, if not superior, to that of CDDP and ADM. Against MX-1, it was more effective than either ADM or CDDP and cured one of six mice at a dose of 100 mg/kg. MMC showed the highest activity but demonstrated chronic toxicity, resulting in a ΔBW_{max} value of 8.9% on day 28, whereas CPT-11 temporarily caused a slight reduction in body weight (ΔBW_{max}, 1.2% on day 5). CPT-11 was significantly more effective than ADM against St-15. Temporary suppression of the growth of SC-6 was observed after a single i. v. injection of CPT-11, ADM, and CDDP, with IR_{max} values of 51.5%, 46.5%, and 76.9%, respectively, being recorded. In all tests mentioned above, the ΔBW_{max} value for mice receiving CPT-11 was lower than that for animals given either ADM, CDDP, or MMC.

Effects of CPT-11 on human tumor xenografts after three i. v. injections

As shown in Table 2, the antitumor activity of CPT-11 given i. v. every 4 days for a total of three doses (q4d × 3) was greater than that seen after a single i. v. injection. At total doses of 100 and 200 mg/kg, respectively, CPT-11 cured 4/6 and 6/6 mice inoculated with MX-1. At a dose of 200 mg/kg, it was significantly more effective than ADM against MX-1, and the mice showed no loss of body weight (ΔBW_{max}, −1.1%).

Against St-15 as well, CPT-11 exhibited antitumor activity significantly higher than that of ADM, yielding IR_{tw} values of 77.8% and 94.4% at total doses of 100 and 200 mg/kg, respectively. Statistically significant effects of this agent were demonstrated at a total dose of 200 mg/kg on SC-6 and QG-56, the IR_{tw} values being 80% and 56.6%, respectively. In tests using three i. v. injections, CPT-11 induced little, if any, reduction in body weight (ΔBW_{max}, <4%), yielding IR_{max} values of >85%, although ADM caused major body weight losses (ΔBW_{max}, ca. 20%).

Effects of CPT-11 given p. o. on human tumor xenografts

Oral administration of CPT-11 was also effective against some human tumor xenografts, as summarized in Table 3. When mice were treated p. o. with CPT-11 three times (q4d × 3) at total doses that were 2–4 times higher than those used for i. v. administration, the effects were approximately equal, if not superior, to those obtained using the latter route.

Treatments with a total dose of 800 mg/kg resulted in strong antitumor activity against Co-4, St-15, and QG-56,

Table 2. Comparison of the antitumor activity of CPT-11 vs ADM after three i.v. injections

Tumor	Agent	Total dose (mg/kg)	IR_{max}[a] (%)	IR_{tw}[b] (%)	ΔBW_{max}[c] (%)	Cured mice/ total[d]
MX-1	CPT-11	50	75.9 (12)	48.6	−2.1 (8)	0/6 (30)
		100	100 (20)	99.7**	−1.7 (2)	4/6
		200	99.8 (20)	100 **,***	−1.1 (2)	6/6
	ADM	10	28 (20)	11.4	0 (12)	0/6
		20	79.9 (20)	65.4	20.6 (12)	0/6
St-15	CPT-11	100	73.1 (20)	77.8**	−2 (10)	0/6 (30)
		200	86.2 (30)	94.4**,***	2.2 (10)	0/6
	ADM	10	46.3 (20)	47.2	2.5 (10)	0/6
		20	61 (27)	68 *	18.3 (14)	0/6
SC-6	CPT-11	100	71.1 (14)	56.3*	−0.4 (2)	0/6 (28)
		200	88.8 (14)	80 **	3.6 (6)	0/6
	ADM	10	44.6 (21)	42.4	3.6 (4)	0/6
		20	74.6 (28)	69.5**	20.1 (11)	0/6
QG-56	CPT-11	200	86.1 (14)	56.6**	3 (2)	0/6 (27)
	ADM	20	5.1 (22)	28.7	19 (14)	0/6

Nude mice were treated i.v. every 4 days for a total of 3 injections beginning from day 0

[a] Maximal tumor growth-inhibition rate calculated from the estimated tumor volume; numbers in parentheses indicate the days on which IR_{max} was reached after initial drug administration

[b] Tumor growth-inhibition rate calculated from the tumor weight: * $P < 0.05$, ** $P < 0.01$ as compared with untreated controls; *** $P < 0.05$ as compared with ADM at 20 mg/kg

[c] Maximal rate of body weight reduction; numbers in parentheses denote the days of ΔBW_{max}

[d] Numbers in parentheses indicate the days on which the tumor weight and number of mice cured were measured

Table 3. Comparison of the antitumor activity of CPT-11 vs other anticancer agents after p.o. administrations

Tumor	Agent	Total dose (mg/kg)	IR_{max}[a] (%)	IR_{tw}[b] (%)	ΔBW_{max}[c] (%)	Cured mice/ total[d]
Co-4	CPT-11	400	92.9 (20)	89.8*	–	0/6 (28)
		800	97.9 (26)	98 *,***	–	0/6
	FT	1200	70.4 (28)	69.1	–	0/6
MX-1	CPT-11	200	100 (19)	99.4*,**	0.9 (6)	2/6 (28)
		400	100 (19)	100 *,***	1.7 (11)	6/6
		800	100 (19)	99.8*,***	6.6 (11)	5/6
	FT	1200	34.5 (15)	24.7	2.3 (6)	0/6
St-15	CPT-11	800	88.9 (28)	87.8*,**	12.7 (12)	0/5 (28)
	FT	1200	53 (28)	35	1.2 (12)	0/5
QG-56	CPT-11	400	90.5 (13)	74.3*,**	6 (7)	0/6 (27)
		800	98.3 (17)	94.1*,***	10.8 (7)	0/6
	5-FU	100	18.1 (17)	7.2	3 (7)	0/6

Nude mice were treated p.o. every 4 days for a total of three doses beginning from day 0

[a] Maximal tumor growth-inhibition rate calculated from the estimated tumor volume; numbers in parentheses indicate the days on which IR_{max} was reached after initial drug administration

[b] Tumor growth-inhibition rate calculated from the tumor weight: * $P < 0.01$ as compared with untreated controls; ** $P < 0.05$, *** $P < 0.01$ as compared with FT or 5-FU

[c] Maximal rate of body weight reduction in mice; numbers in parentheses denote the days of ΔBW_{max}. −, Not determined because of the body weight loss in the untreated control

[d] Numbers in parentheses indicate the days on which the tumor weights and the number of cured mice were measured

giving respective IR_{tw} values of 98%, 87.8%, and 94.1%. Furthermore, 2/6, 6/6, and 5/6 mice bearing MX-1 tumors were cured at total doses of 200, 400, and 800 mg/kg, respectively. Oral administration induced moderate body weight loss in mice, with ΔBW_{max} values of <13% being recorded. Against the four human tumors Co-4, MX-1, St-15, and QG-56, CPT-11 was significantly more effective than either FT or 5-FU after three oral treatments.

Effect of CPT-11 given daily p.o. on MX-1

Table 4 compares the effect of CPT-11 on MX-1 following daily oral administration for 10 days with that after a single i.v. injection at the same total dose of 100 mg/kg. Under such conditions, the IR_{tw}, IR_{max}, and ΔBW_{max} values obtained after p.o. administration were lower than those resulting from i.v. injection. Temporary tumor regression was induced after i.v. treatment, and a cytostatic effect, reflected by an IR_{max} value of 61.4%, was observed on oral

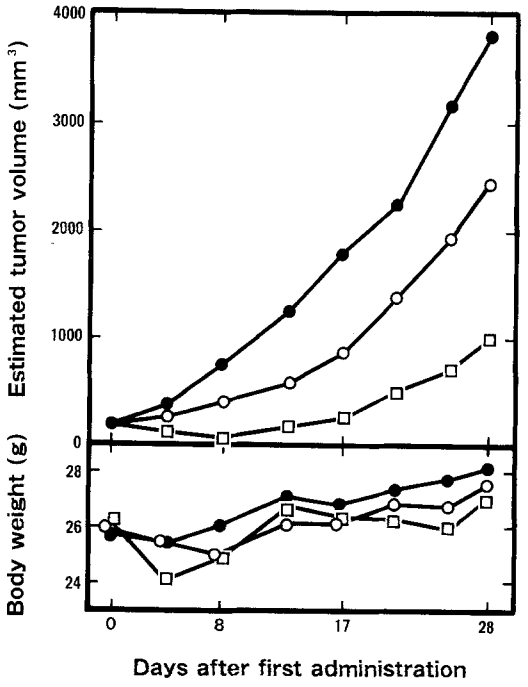

Fig. 2. Changes in the volumes of MX-1 tumor xenografts and in the body weights of mice after treatments with CPT-11. Fragments of MX-1 tumor were transplanted s.c. into nude mice. When the estimated tumor volume had reached the range of 100–300 mm³, mice were treated either with no agent (●) or with CPT-11 given at a single i.v. dose of 100 mg/kg (□) or given orally for 10 consecutive days at a total dose of 100 mg/kg (○)

Table 4. Comparison of the activity of CPT-11 against MX-1 on a single i.v. injection vs on daily p.o. treatment

Route	Schedule	Total dose (mg/kg)	IR_{max}[a] (%)	IR_{tw}[b] (%)	ΔBW_{max}[c] (%)
p.o.	qd ×10	100	61.4 (13)	38.4	3.9 (7)
i.v.	qd × 1	100	94.3 (8)	70.8*	8.1 (1)

[a] Maximal tumor growth-inhibition rate calculated from the estimated tumor volume; numbers in parentheses indicate the days on which IR_{max} was reached after the first treatment on day 0

[b] Tumor growth-inhibition rate determined from the tumor weight on day 28. The *asterisk* indicates a statistically significant difference from the untreated control value (* $P < 0.05$)

[c] Maximal rate of body weight reduction; numbers in parentheses denote the days of ΔBW_{max}

Table 5. In vitro and in vivo antitumor activity of CPT-11 and SN-38 against QG-56 cells

Agent	In vitro: IC_{50}[a] (μg/ml)	In vivo[b]			
		Dose (mg/kg)	IR_{max} (%)	IR_{tw} (%)	ΔBW_{max} (%)
CPT-11	3.96	100	24.9	4	1.7
SN-38	0.00195	200	37.1	23.8	0

[a] Concentration of each agent inducing 50% inhibition of cell growth (by MTT assay)

[b] Nude mice were given a single i.v. dose of each agent (5 mice/group) IR_{max}, Maximal tumor growth-inhibition rate calculated from the estimated tumor volume; IR_{tw}, tumor growth-inhibition rate calculated from tumor weight on day 28; ΔBW_{max}, maximal rate of body weight reduction

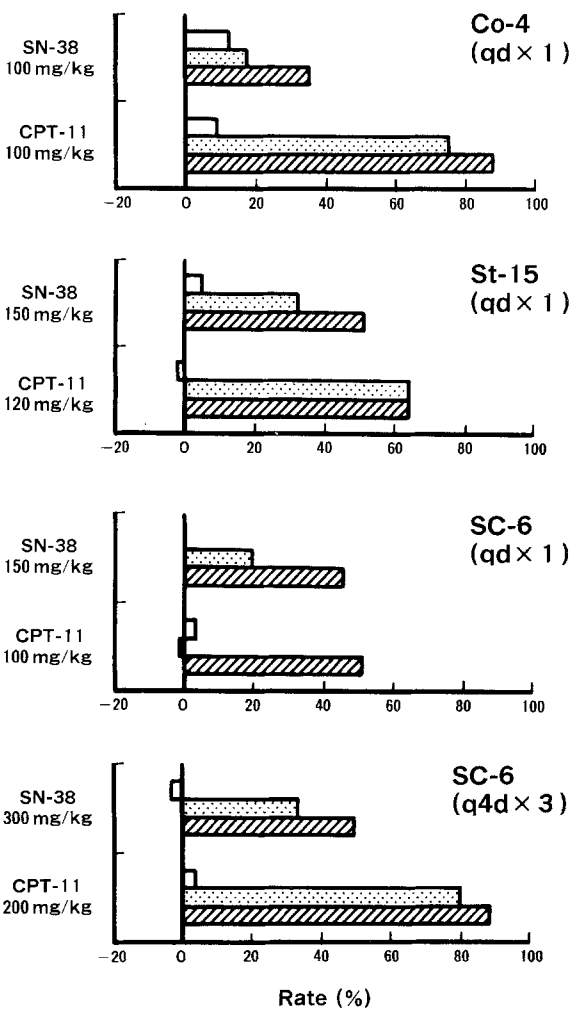

Fig. 3. Antitumor effects of CPT-11 and SN-38 on Co-4, St-15, and SC-6 tumors as evaluated according to the maximal tumor growth-inhibition rate calculated from the estimated tumor volume (IR_{max}, ▨), to the tumor growth-inhibition rate calculated from tumor weight (IR_{tw}, □), and to the maximal rate of body weight reduction (ΔBW_{max}, □). When the estimated tumor volume had reached 100–300 mm³ in nude mice, the animals received CPT-11 and SN-38 at respective single i.v. doses of either 100 or 120 mg/kg and either 100 or 150 mg/kg, or they were given CPT-11 or SN-38 every 4 days for a total of 3 i.v. injections at respective total doses of 200 and 300 mg/kg (SC-6)

treatment during the period of administration. Tumor regrowth occurred at almost the same rate in the untreated control after the period of oral treatment, as is shown in Fig. 2.

Comparison of the antitumor activity of CPT-11 and SN-38

The IC_{50} values for CPT-11 and SN-38, a metabolite of the former, against QG-56 cells were 4 μg/ml and 2 ng/ml, respectively (Table 5). Against QG-56, however, the antitumor activity of a single i.v. dose of 200 mg/kg SN-38 was similar to that of 100 mg/kg CPT-11. As demonstrated in Fig. 3, the antitumor effects (IR_{max} and IR_{tw} values) of SN-38 on Co-4 and St-15 were inferior to those of CPT-11 after a single i.v. injection, with the ΔBW_{max} values for the former being greater than those for the latter. Moreover,

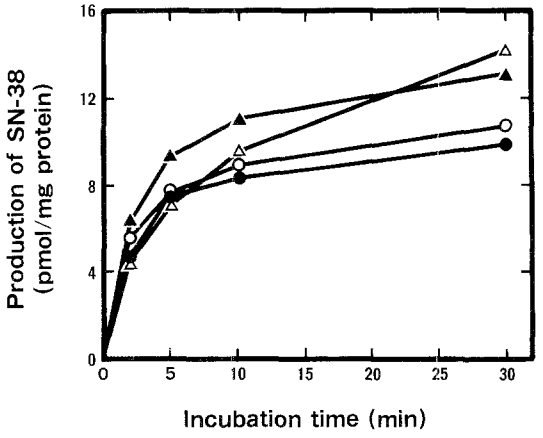

Fig. 4. Amount of SN-38 produced from CPT-11 by homogenates of Co-4 (○), MX-1 (●), SC-6 (△), and QG-56 (▲) tumors. The reaction was initiated by the addition of CPT-11 to the tumor homogenate and was terminated after incubation for 2, 5, 10, and 30 min at 37° C. The amount of SN-38 in the mixture was determined by fluorospectrometry

after three i.v. treatments at twice the dose of a single injection, the antitumor effect of CPT-11 on SC-6 was clearly enhanced, but that of SN-38 remained unchanged.

Relationship between the antitumor effect of CPT-11 and the production of SN-38 from CPT-11 by tumors

Figure 4 illustrates that each homogenate of Co-4, MX-1, SC-6, and QG-56 demonstrated the same ability to produce SN-38 from CPT-11. During the first 5 min, the homogenate produced SN-38 at rates of 1.4–1.9 pmol /mg protein^{-1} min^{-1}. The rates were reduced to values of <0.5 pmol/mg protein^{-1} min^{-1} after 30 min incubation.

Discussion

CPT-11, a derivative of camptothecin (CPT), has shown antitumor activity not only against ascites tumors, including sublines resistant to ADM and to vincristine, but also against solid tumors implanted into mice [14, 22]. Furthermore, this compound has effectively inhibited experimental lung metastasis of highly metastatic murine tumors [16].

The present study demonstrated that either i.v. or p.o. administration of CPT-11 was significantly effective against human tumor xenografts transplanted into nude mice. CPT-11 injected i.v. either once or every 4 days for a total of three treatments showed antitumor activity higher than that of ADM against five human tumors: colon adenocarcinoma Co-4, mammary carcinoma MX-1, gastric adenocarcinomas St-15 and SC-6, and squamous-cell lung carcinoma QG-56. Therefore, CPT-11 may be a clinically effective anticancer drug when given i.v. A recent clinical study revealed that it was indeed effective against non-small-cell lung cancers, including squamous-cell lung carcinoma [4].

After i.v. injections, CPT-11 caused a smaller maximal rate of body weight reduction in mice (ΔBW_{max}) than did either ADM, CDDP, or MMC. Moreover, the interval between the day of the first i.v. injection and the day on which ΔBW_{max} occurred was shorter than or equal to those found for control drugs, as shown in Tables 1 and 2, and the body weights of the mice soon recovered, as illustrated in Fig. 2. These observations suggest that the toxicity of CPT-11 is lower than that of other anticancer drugs, such as ADM, and is not chronic.

Three (q4d × 3) i.v. injections of CPT-11 gave higher values for both IR_{max} and IR_{tw} and smaller values for ΔBW_{max} than those obtained following a single i.v. injection at the same total dose of 100 mg/kg. These data mean that a schedule of intermittent injections is superior to a single injection both in antitumor activity and in safety; thus, the effect of CPT-11 depends on the schedule of administration. It is known that mammalian cells in the S-phase show the highest sensitivity to CPT [3, 15]. Recent studies have demonstrated that CPT exerts an inhibitory effect on type I DNA topoisomerase [9, 10] and suggest that the inhibition of type I DNA topoisomerase in S-phase cells by CPT causes the arrest of replication forks and results in cell killing [12]. The S-phase-specific effect of CPT may offer a possible reason for the schedule-dependent antitumor activity of CPT-11, as demonstrated in other S-phase-specific drugs [21].

CPT-11 given p.o. three times (q4d × 3) was also highly active and was significantly more effective than either FT or 5-FU. The oral route of administration delivered doses of CPT-11 that were 4 times those given by the i.v. route according to the same schedule. The effect of oral treatment with CPT-11 at the maximal dose was equal, if not superior, to that of i.v. injection, as demonstrated in Tables 2 and 3. Especially against MX-1, three oral doses resulted in very strong antitumor activity. These results suggest that oral treatment with CPT-11 has potential as adjuvant chemotherapy similar to that using FT or 5-FU. Table 4 shows that daily oral treatment at low doses may be useful in adjuvant chemotherapy, because of the cytostatic effect and low toxicity obtained.

It was recently reported that SN-38, a metabolite of CPT-11, was much more cytotoxic in vitro [13] but less effective on murine tumors in vivo [14] than was CPT-11. In the present study, we also found that SN-38 was much more cytotoxic to human tumor cells but that its effects on solid human tumors in nude mice were similar, if not inferior, to those of CPT-11. Kaneda et al. [13] demonstrated that the plasma concentration of SN-38 in mice was maintained longer after CPT-11 administration than after treatment with SN-38 and suggested that the maintenance of SN-38 levels in plasma was necessary for the superior antitumor activity of CPT-11. These authors also observed that SN-38 was produced from CPT-11 in mouse serum and tissue (liver and small intestinal mucosa) homogenate [13].

If the effect of CPT-11 depends on the production of SN-38, the ability of human tumors to produce SN-38 from CPT-11 must be examined. As shown in Fig. 4, this ability was almost equal in tumors that were highly sensitive to CPT-11 (Co-4 and MX-1) and in those showing low CPT-11 sensitivity (SC-6 and QG-56). Under the same conditions, plasma and tissue (liver, kidney, intestine, and lung)

homogenate prepared from nude mice converted CPT-11 to SN-38 more efficiently than did these tumors (unpublished observations). Therefore, the effect of CPT-11 on each tumor seems to be independent of the production of SN-38 in the tumor but may depend on SN-38 produced in tissue and/or plasma.

In conclusion, CPT-11 exhibits potent antitumor activity against human tumor xenografts. This compound is therefore expected to be clinically effective if its dose and the schedule of administration are suitably controlled, and phase II clinical trials in Japan are revealing its efficacy against malignancies other than lung cancer.

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