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Antitumor Activity of 7-Ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxy-camptothecin, a Novel Water-soluble Derivative of Camptothecin, against Murine Tumors[1]

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ABSTRACT

The search for new water-soluble analogues of camptothecin (CPT) with higher activity and less toxicity has led to the development of a novel compound, 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxy-camptothecin (CPT-11), which showed significant antitumor activity against a broad spectrum of experimental tumor models by i.p., i.v., or oral administration. When its activity against L1210 was compared with that of CPT and known derivatives, CPT-11 was most effective, giving the highest maximum increase in life span (ILS) and showing good activity over a wide dose range. The antitumor activity of CPT-11 was shown against tumors not only in the ascites form but also in the solid form. Included among the more susceptible murine tumors are S180, Meth A fibrosarcoma, Lewis lung carcinoma, Ehrlich carcinoma, MH134 hepatoma, mammary carcinoma of C3H/HeN mice, L1210, and P388 leukemia. Probable cures of these tumors were induced frequently by CPT-11. The antitumor activity of CPT-11 against i.p.-implanted L1210 was superior to that of Adriamycin in maximum ILS, the number of cured mice, and the therapeutic ratio. CPT-11 at a dose of 100 mg/kg produced an ILS in excess of 300% with five of six mice surviving tumor free, and effected 100% tumor regression at 200 mg/kg, whereas the optimum dose of Adriamycin, 12.5–25 mg/kg, brought about 114–129% ILS with one of six mice surviving. The acute toxicity of CPT-11 was extremely low, particularly in the case of oral administration. CPT-11 is expected to be clinically useful.

INTRODUCTION

CPT[3] (Fig. 1) is a plant antitumor agent which was isolated from *Camptotheca acuminata*, a tree native to south China by Wall *et al.* (1). It has a very good spectrum of activity against experimental animal tumor models in ascites as well as solid form but its application as a therapeutic has been hampered by severe toxicity. Clinical and preclinical studies revealed reversible bone marrow depression and hemorrhagic cystitis as the major dose-limiting toxicity (2, 3).

Efforts have been directed at finding new derivatives of CPT with higher antitumor activity and less toxicity (for review, see Ref. 4). For example, 10-hydroxy-CPT has been reported to have almost the same antitumor activity as CPT with less toxicity (4). We prepared many partially synthesized CPT derivatives including 7-ethyl-CPT (Fig. 1, *SN-22*) and 7-ethyl-10-hydroxy-CPT (Fig. 1, *SN-38*) having strong activity against various murine tumors (5). However, SN-22 and most of the derivatives synthesized previously by us or other investigators as well as CPT itself are not soluble in water. Poor aqueous solubility posed a number of problems in their clinical application. Alkaline treatment of the compounds resulted in water-soluble sodium salt forms with the δ-lactone ring opened. Since it was pointed out that the hydroxy lactone ring in CPT is absolutely required for its antitumor activity (4), water-soluble derivatives having an intact δ-lactone ring were investigated. Among them, 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxy-camptothecin (CPT-11) showed marked activity against mouse leukemia L1210, with little toxicity.

The present work was undertaken to evaluate the effect of CPT-11 administered through diverse routes on various kinds of murine tumors both in ascites and in solid forms. The activity was compared with that of CPT and its known derivatives, or current clinically useful anticancer agents including Adriamycin. The acute toxicity of CPT-11 for experimental animals was also quantitated. Portions of this work have been presented in preliminary form (6).

MATERIALS AND METHODS

Animals and Tumors. An appropriate strain of SPF adult mice for each tumor system was obtained commercially (Table 1). All tumors used were maintained in our laboratory by serial transplantation.

Antitumor Testing. The route and size of the tumor inoculum and host mouse used in each experiment are summarized in Table 1. Mice were inoculated with the appropriate number of viable tumor cells i.p. or s.c. on day 0. CPT-11 was dissolved in sterile physiological saline immediately before use and administered to mice i.p., i.v., or p.o. on days 1, 5, and 9, and also on days 13 and 17 for B16 melanoma. When the activity of CPT-11 against L1210 was evaluated concomitantly with that of Adriamycin, 5×10^5 cells were inoculated i.p. into CDF_1 mice on day 0 and the drugs were administered i.p. on days 1–9. The activity of CPT-11 was determined in parallel with that of 5FU against Sarcoma 180 (S180), 2×10^6 cells being inoculated s.c. on day 0 and drugs being administered p.o. on days 1, 5, and 9. The activity of CPT-11 was compared with that of CPT and derivatives against L1210, 5×10^5 cells being implanted i.p. into CDF_1 on day 0 and the compounds being injected i.p. on days 1–5.

Evaluation of Antitumor Activity. For the ascites type of tumor the percentage of ILS was calculated by the following formula for comparison:

$$\text{ILS (\%)} = (T/C - 1) \times 100 \quad \text{(A)}$$

where T and C are the survival periods for treated and control mice, respectively. The survival times were compared by employing the generalized Wilcoxon test. ILS 30% or T/C 130% was adopted for the criteria of therapeutic efficacy against ascites type of tumor. Longer survivors than 40 days in L1210 and P388 and 60 days in the other ascites tumors with no retention of ascites were considered to be cured. Solid tumors were extirpated and weighed on day 21, and the tumor IR was calculated by the following formula:

$$\text{IR (\%)} = (1 - T/C) \times 100 \quad \text{(B)}$$

where T and C are the tumor weight in treated and control mice, respectively. Student's t test was used to assess the statistical signifi-

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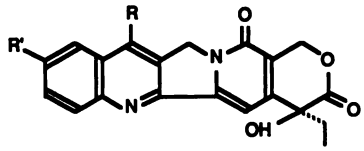
[3] The abbreviations used are: CPT, camptothecin; 5FU, 5-fluorouracil; ILS, increase in life span; IR, inhibition ratio; TR, therapeutic ratio; LD_{50}, 50% lethal dose.



	R	R'
Camptothecin	H	H
SN-22	Ethyl	H
10-Hydroxycamptothecin	H	OH
SN-38	Ethyl	OH
CPT-11	Ethyl	OCO-N(piperidine)-N(piperidine)·HCl

Fig. 1. Structure of camptothecin and derivatives.

Table 1 *Details of experimental protocol*

Tumor[a]	Mouse		Inoculum		Drug treatment	
	Strain	Age[b]	No. of cells	Site	Schedule[c]	Route
L1210	CDF1	7 w	5×10^5	i.p.	d 1,5,9	i.p.,i.v.,p.o.
P388	CDF1	7 w	2×10^6	i.p.	d 1,5,9	i.p.,i.v.,p.o.
S180	ICR	7 w	2×10^6	i.p.	d 1,5,9	i.p.,p.o.
			2×10^6	s.c.	d 1,5,9	i.v.,p.o.
Meth A	BALB/c	7 w	2×10^6	i.p.	d 1,5,9	i.p.,p.o.
			5×10^5	i.p.	d 1,5,9	p.o.
			5×10^5	s.c.	d 1,5,9	i.v.,p.o.
B16	C57BL/6	7 w	2×10^6	i.p.	d 1,5,9,13,17	i.p.,i.v.,p.o.
Adenoca. 755	BDF1	7 w	2×10^6	s.c.	d 1,5,9	i.p.,p.o.
Lewis lung ca.	BDF1	7 w	1×10^6	s.c.	d 1,5,9	i.p.,p.o.
Mammary ca.	C3H/HeN	8 w	2×10^6	s.c.	d 1,5,9	i.p.,p.o.
Ehrlich ca.	ICR	7 w	2×10^6	i.p.	d 1,5,9	i.p.,p.o.
			2×10^6	s.c.	d 1,5,9	i.p.,p.o.
MH134	C3H/HeN	8 w	2×10^6	i.p.	d 1,5,9	i.p.
			2×10^6	s.c.	d 1,5,9	i.p.,p.o.

[a] Adenoca., adenocarcinoma; ca., carcinoma.
[b] w, weeks.
[c] d, day.

cance of the observed differences between mean tumor weights. IR, 58% or T/C, 42% was employed for the efficacy criteria in the case of solid type of tumor (7). The mice without any trace of tumor at the termination of the experiments were considered to be cured. The TR was calculated by the following formula:

$$TR = \text{dose for ILS maximum/dose for ILS 30\%} \qquad (C)$$

Toxicity Testing. Male 7-week-old ICR mice were used for the toxicity evaluation. Mice in groups of 10/dose were treated by single i.p. injection of CPT-11. LD_{50} values were calculated by the method of Litchfield-Wilcoxon. Similarly, the toxicity test was performed using male and female 6-week-old Fisher rats.

Materials. CPT-11 was synthesized by chemical modification of camptothecin, introducing an ethyl group at the 7-position (SN-22), then a hydroxyl group at the 10-position (SN-38) via [N^{14}]oxide of SN-22 (8, 9) and then binding a piperadinopiperadinocarbonyl group to the hydroxyl group (9, 10).

RESULTS

Antitumor Activity of CPT-11 against Ascites Tumors. The i.p. administration of CPT-11 prolonged the survival time of the L1210- (Table 2) or P388-bearing mice at total doses of 6.3 mg/kg or more and many mice were cured of the ascites tumors at total doses of 100–400 mg/kg. Against the ascites form of S180 (Table 3), Meth A and Ehrlich carcinoma, the i.p. administration was effective at total doses of less than 3.13 mg/kg and many mice survived longer than 60 days tumor free at total doses of 6.3–400 mg/kg for S180 and Ehrlich and of 50–400 mg/kg for Meth A. Particularly, at total doses of 25–200 mg/kg for S180, and of 200–400 mg/kg for Meth A, all the mice escaped death from the tumor. The ascites form of MH134 was as sensitive to i.p. treatment with CPT-11 as Ehrlich ascites carcinoma. The p.o. administration of CPT-11 also induced a

Table 2 *Antitumor activity of CPT-11 administered i.p. against i.p.-implanted leukemia L1210*

Total dose (mg/kg)	Survival time (days, mean ± SD)[a]	T/C[a] (%)	Survivors on day 40
0	7.2 ± 0.4	(100)	0/6
3.13	8.3 ± 0.5	116	0/6
6.25	9.5 ± 0.5	132	0/6
12.5	13.3 ± 0.5	185	0/6
25	16.7 ± 1.6	231	0/6
50	20.8 ± 1.8	289	0/6
100	27.4 ± 5.1	381	1/6
200	29.0	403	5/6
400	28.5	396	4/6

[a] The mean, SD, and T/C were determined for nonsurvivors only.

Table 3 *Antitumor activity of CPT-11 administered i.p. against i.p.-implanted Sarcoma 180*

Total dose (mg/kg)	Survival time (days, mean ± SD)[a]	T/C[a] (%)	Survivors on day 60
0	12.8 ± 3.1	(100)	0/6
3.13	27.3 ± 8.7	214	0/6
6.25	37.5 ± 4.2	293	2/6
12.5	39.3 ± 7.7	307	3/6
25			6/6
50			6/6
100			6/6
200			6/6
400	8.0 ± 2.9	63	2/6

[a] Tumor-free survivors were excluded from calculations of the mean, SD, and T/C (%).

Table 4 *Evaluation of antitumor activity of CPT-11 against i.p.-implanted tumors*

Tumor	Drug route	ILS max %	Total dose (mg/kg)		Therapeutic ratio
			ILS max	ILS 30%	
L1210	i.p.	300	400–200	6	67–33
	i.v.	≥186	≥200	12.5	≥16
	p.o.	≥200	≥800	70	≥11
P388	i.p.	310	200	2.5	80
	i.v.	≥170	≥200	10	≥20
	p.o.	≥172	≥800	50	≥16
S180	i.p.	≥370	200	<3	>67
	p.o.	≥292	≥800	25	≥32
Meth A	i.p.	≥388	≥400	2	≥200
	p.o.	≥216	≥800	130	≥6
B16	i.p.	≥86	≥400	45	≥9
	i.v.	≥43	≥400	200	≥2
	p.o.	≥56	≥1600	800	≥2
Ehrlich	i.p.	≥340	200	<3	>67
	p.o.	≥300	600	60	10
MH134	i.p.	≥124	200	6	33

significant therapeutic response in curing mice of the following tumors in ascites form: P388 and MH134, cure in two mice out of six at a total dose of 800 mg of CPT-11/kg; Ehrlich and S180, more than five out of six cured at 200–800 mg/kg. The i.v. treatment was effective against L1210, P388, and B16; however, CPT-11 administered by the i.v. route was two to four times less active than by the i.p. route. High doses of CPT-11 were inhibitory to B16 by any route of administration. The antitumor activity of CPT-11 in several tumor models in ascites form is summarized in Table 4.

Antitumor Activity of CPT-11 against Solid Tumor. CPT-11 demonstrated strong tumor-inhibiting activity against the following tumors in solid form: Lewis lung carcinoma (Fig. 2), Ehrlich, MH134, S180, and Meth A. The activity was induced by any of the administration routes i.p., i.v., or p.o. The effects of CPT-11 on the solid form of tumors are summarized in Table 5. As seen in the table, CPT-11 treatment frequently resulted in tumor-free mice (complete inhibition of the tumor growth) at total doses of 200 mg/kg or above, or at 50–100 mg/kg in the case of Ehrlich, when administered i.p. or i.v. and at 400 mg/kg or above by the p.o. route. It showed moderate

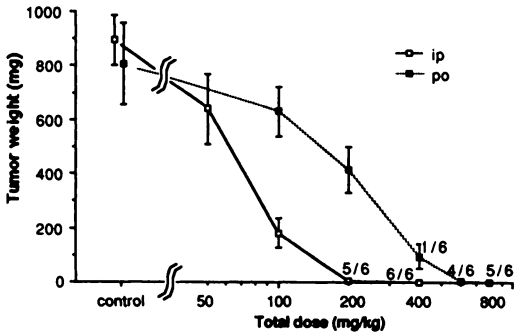


Fig. 2. Antitumor activity of CPT-11 administered i.p. or p.o. against s.c.-implanted Lewis lung carcinoma. *Points*, mean tumor weight; *bars*, SE; *numbers next to points*, number of tumor-free mice/number of tested mice. Tumor-free mice were excluded from the calculation of the mean and SE.

Table 5 *Evaluation of antitumor activity of CPT-11 against s.c.-implanted tumors*

Tumor	Drug route	Total dose (mg/kg)[a] ID_{58}	Total dose (mg/kg)[a] ID_{90}	Dose for cure[b] (mg/kg)	No. of cured mice/total (dose, mg/kg)
S180	i.v.	50	180	200	4/10 (200)
	p.o.	100	365	400	3/10 (400), 6/10 (800)
Meth A	i.v.	61	195	>400	6/10 (1,000)
	p.o.	140	420	1000	7/7 (1,600)
Lewis lung ca.	i.p.	72	118	200	5/6 (200), 6/6 (400)
	p.o.	215	410	400	1/6 (400), 4/6 (600), 5/6 (800), 6/6 (1,600)
Ehrlich	i.p.	32	92	50	1/6 (50), 3/6 (100), 3/6 (200)
	p.o.	110	450	400	2/6 (400), 3/5 (800)
MH134	i.p.	66	200	200	1/6 (200), 1/6 (400)
	p.o.	215	620	600	1/6 (600), 4/6 (800)
Mammary ca.	i.p.	96	>400	200	1/6 (200)
	p.o.	600	>800	800	1/6 (800)

[a] Total dose for 58% or 90% inhibition of tumor growth. The values were obtained graphically on a probit scale. Tumor-free mice were excluded from the calculation of 90% inhibition.
[b] The minimum total dose for incidence of total regression of the tumor.

activity against mammary carcinoma of C3H mice. CPT-11 was not effective against adenocarcinoma 755 under the present conditions.

Comparison of the Activity of CPT-11 and CPT and Derivatives. The antitumor activity of CPT-11 against i.p.-implanted L1210 was evaluated in parallel with that of CPT and the known derivatives SN-22 and SN-38 as shown in Fig. 3. Among those compounds, CPT-11 gave the highest *T/C* (%) and the widest dose range where the effect was significant. Fig. 3 also includes the activity of the corresponding sodium salt. It is clearly indicated that the sodium salt forms have greatly decreased antileukemic activity compared to the original compounds.

Comparison of the Activity of CPT-11 with That of Current Clinically Useful Anticancer Agents. Table 6 compares the efficacy of CPT-11 and Adriamycin in the treatment of i.p.-implanted L1210. CPT-11 at a total dose of 100 mg/kg produced an ILS in excess of 300% (*T/C*, 414%) with five of six mice surviving tumor free and effected 100% tumor regression at 200 mg/kg, whereas the optimum dose of Adriamycin, 12.5–25 mg/kg, brought about 114–129% ILS with one of six mice surviving. Table 7 compares the efficacy of CPT-11 and 5FU in the treatment of s.c.-implanted S180. At a total dose of 400 mg/kg, CPT-11 cured the tumor in three out of 10 mice, whereas no tumor-free mice were observed after treatment by 5FU even at such a high dose as caused death of toxicity in five of 10 mice, and at 800 mg/kg, CPT-11 induced a cure in six

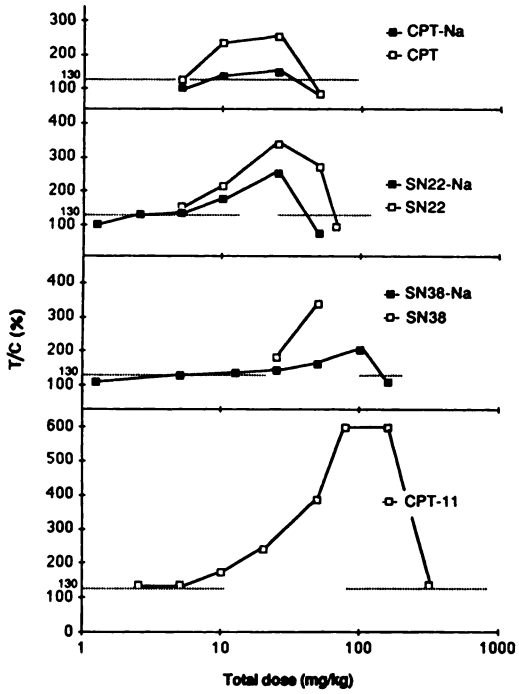


Fig. 3. Comparison of the antitumor activity of CPT and derivatives against i.p.-implanted L1210 leukemia. L1210 cells (5×10^5/mouse) were inoculated i.p. into CDF_1 mice on day 0 and the drugs were administered i.p. on days 1–5.

out of 10 mice whereas the mice treated with 5FU exhibited 100% mortality by day 21.

Acute Toxicity of CPT-11. Acute toxicity of CPT-11 was evaluated after single administration by the i.p. and p.o. routes and compared with that of CPT. As shown in Table 8, the LD_{50} values of 117.5 and 765.3 mg/kg by the i.p. and p.o. routes, respectively, are much higher than those of CPT. A similar acute toxicity test showed that LD_{50} values of CPT-11 in rats were 83.6 (male) and 85.1 (female) mg/kg by i.v. injection and 866.9 (male) and 1026.5 (female) mg/kg by p.o. administration.

DISCUSSION

Camptothecin (CPT) and most of its known derivatives such as 7-ethyl-CPT (SN-22), 10-hydroxy-CPT, and 7-ethyl-10-hydroxy-CPT (SN-38) are not sufficiently soluble in water for clinical use. Alkaline treatment of these compounds yielded water-soluble sodium salt forms with the δ-lactone ring opened and diminished activity. The results are in general agreement with the proposal of Wall *et al.* that the hydroxy lactone ring in CPT is absolutely required for its antitumor activity (4).

The search for new water-soluble derivatives having an intact δ-lactone ring had led to the development of a novel compound, CPT-11, which demonstrated significant antitumor activity against a broad spectrum of experimental tumor models. Comparing the activity of CPT-11 against L1210 with that of CPT and its derivatives SN-22, SN-38, and 10-hydroxy-CPT, revealed that CPT-11 was the most effective, giving the highest maximum ILS and showing good activity over a wide dose range. The antitumor activity of CPT-11 was shown against the tumors not only in the ascites form but also in the solid form. Among the more susceptible murine tumors are S180, Meth A fibrosarcoma, Lewis lung carcinoma, Ehrlich carcinoma, MH134 hepatoma, mammary carcinoma of C3H/HeN mice, L1210 leukemia, and P388 leukemia. Probable cures of these tumors were observed frequently after CPT-11 treatment. Al-

Table 6 *Comparison of the antitumor activity of CPT-11 and Adriamycin against i.p.-implanted L1210*

L1210 cells (5×10^5/mouse) were inoculated i.p. into CDF_1 mice on day 0 and the drugs were administered i.p. on days 1–9.

	CPT-11			Adriamycin		
Total dose (mg/kg)	Survival time (days, mean ± SD)	*T/C* (%)	Survivors on day 40	Survival time (days, mean ± SD)	*T/C* (%)	Survivors on day 40
Control	7.0 ± 0.0	100	0			
0.78	8.5 ± 0.5	121	0	8.8 ± 0.4	126	0
1.56	9.0 ± 0.0	129	0	9.7 ± 0.5	138	0
3.13	9.7 ± 0.5	138	0	10.7 ± 0.5	152	0
6.25	11.2 ± 0.4	160	0	11.7 ± 0.7	167	0
12.5	13.8 ± 0.4	198	0	16.0 ± 3.7	229	0
25	15.8 ± 0.7	226	0	15.0 ± 0.9[a]	214	1/6
50	18.8 ± 1.6	269	0	10.0 ± 0.0	143	0
100	29.0[a]	414	5/6	6.3 ± 0.5	90	0
200			6/6	5.0 ± 0.0	71	0

[a] Tumor-free survivors were excluded from calculations of the mean, SD and *T/C* (%).

Table 7 *Comparison of the antitumor activity of CPT-11 and 5FU against s.c.-implanted S180*

S180 cells (2×10^6/mouse) were inoculated s.c. into ICR mice on day 0 and the drugs were administered p.o. on days 1, 5, and 9. The tumors were excised and weighed on day 21.

Drug	Total dose (mg/kg)	Tumor weight (g, mean ± SD)[a]	Inhibition ratio (%)	Cured mice	Mortality on day 21
Control	0	2.03 ± 0.88		0/10	0/10
CPT-11	200	0.31 ± 0.20	84.5	0/10	0/10
	400	0.12 ± 0.19	94.1	3/10	0/10
	800	0.03 ± 0.05	98.5	6/10	0/10
5FU	200	1.27 ± 0.69	37.4	0/10	0/10
	400	0.38 ± 0.21	81.3	0/10	5/10
	800				10/10

[a] Tumor free mice were excluded from the calculations of the mean and SD.

Table 8 *Acute toxicity of CPT-11 and camptothecin*

LD_{50} was determined by the method of Litchfield-Wilcoxon with 7-week-old male ICR mice.

	LD_{50} (mg/kg)	
Compound	i.p.	p.o.
CPT	56.2 (50.6–62.4)[a]	50.1 (35.5–70.6)
CPT-11	177.5 (131.5–239.6)	765.3 (538.9–1086.7)

[a] Numbers in parentheses, 95% confidence limits.

most all mice bearing S180 or Lewis lung carcinoma were cured of the tumor by the optimal dose of CPT-11.

The antitumor activity of CPT-11 against i.p.-implanted L1210 was superior to that of Adriamycin in the maximum ILS, the number of cured mice, and the therapeutic ratio. CPT-11 at a dose of 100 mg/kg produced an ILS in excess of 300% with five of six mice surviving tumor free and effected 100% tumor regression at 200 mg/kg, whereas the optimum dose of Adriamycin, 12.5–25 mg/kg, brought about 114–129% ILS with one of six mice surviving. Similarly, the therapeutic efficacy of CPT-11 against s.c.-implanted S180 surpassed that of 5FU.

One of the factors which limited the clinical application of CPT was its toxicities, the most prevalent of which is a reversible myelosuppression. The toxicity of CPT-11 was extremely low. The LD_{50} values of CPT-11 administered by various routes were markedly higher than those of CPT. In particular, in oral treatment CPT-11 was 15 times less toxic than CPT. The serial daily i.v. administration of CPT-11 for 4 weeks, however, caused hypoplasia in lymphatic and hematopoietic organs at 20 mg/kg/day for rats and 1.6 mg/kg/day for dogs. Even in these cases, neither cardio-, nephro-, nor pulmotoxicity was recognized. On the occasion of days 1, 5, and 9 administration in mice, the increase in body weight was not affected at a total dose of up to 200 mg/kg by i.p. route or 800 mg/kg by p.o. route. At 400 mg/kg by i.p. route, the weight gain was about 85% of that in the control mice (approximately 2–2.8 g/2 weeks).

The antitumor activity of CPT-11 was shown when it was administered i.v. or p.o. against various tumors. In general, CPT-11 administered by the oral route was 10–20 times less active than by the i.p. route. However, since the toxicity of CPT-11 by the p.o. route was very low, as stated above, it was safely administered p.o. at doses as large as 30 times the effective doses by the i.p. route and induced significant percentages of cure of S180 and Lewis lung carcinoma.

CPT-11 has been selected for development to clinical trial on the basis of its broad spectrum of anticancer activity, potency, and low toxicity. A pharmacological study of CPT-11 is under study and will be published elsewhere.

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