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Inhibition of spontaneous and experimental metastasis by a new derivative of camptothecin, CPT-11, in mice

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Summary. The antimetastatic effect of a new water-soluble derivative of camptothecin, 7-ethyl-10-(4-(1-piperidino)-1-piperidino) carbonyloxy-camptothecin (CPT-11), were examined in several metastatic murine tumor systems. Intravenous (i.v.) injection of CPT-11 into BALB/c mice inhibited lung metastasis by i.v. inoculated, metastatic colonic adenocarcinoma 26 (C26) cells, C26NL-17, in BALB/c mice. This treatment was also effective in C57BL/6 mice against lung metastasis by i.v. inoculated B16-F10 and B16-BL6 cells, highly metastatic variants of the B16 melanoma. Furthermore, intraperitoneal (i.p.) injection of CPT-11 significantly inhibited the growth of C26NL-22 cells, a highly metastatic variant of C26, inoculated subcutaneously (s.c.) into the left front footpads of BALB/c mice. Also, i.p. or i.v. injection of CPT-11 effectively inhibited the growth of 3LL tumors inoculated s.c. into the hind footpads of C57BL/6 mice. Moreover, following s.c. inoculation of either C26NL-22 or 3LL cells, combined surgical excision of the primary tumor and either i.p. or i.v. CPT-11 injections given before or after surgery markedly inhibited the formation of pulmonary metastases. These results show that a new derivative of camptothecin, CPT-11, has a potent inhibitory effect against both spontaneous and experimental lung metastasis.

Introduction

Camptothecin, a plant alkaloid obtained from *Camptotheca acuminata*, was initially isolated in 1966 [18]. Since then there have been many studies into its related substances, and its biological activity has been investigated [1, 3, 7, 10–12, 15, 19]. Camptothecin was found to exhibit a strong antitumor activity against malignant cells, but it has not been clinically used due to its high toxicity [4, 5]. However, its 10-hydroxy derivative has been clinically used in cancer therapy in the People's Republic of China, although it too shows high toxicity. Yokokura et al., using semisynthesis, have prepared many derivatives of camptothecin with reduced toxicity but retaining high antitumor activity [20]. They have reported that i.p., i.v., or orally given 7-ethyl-10-(4-(1-piperidino)-1-piperidino) carbonyloxy-camptothecin (CPT-11), a water-soluble derivative, exhibited a strong antitumor activity against several tumors in murine systems [21]. It has also been reported that in mice CPT-11 not only effectively inhibited tumor growth in both the ascites and solid froms, but also prolonged survival times [9]. Moreover, it could inhibit human tumor growth in vitro and was as effective as other anticancer agents. In the present study, we examined the antimetastatic effect of CPT11 against murine metastatic tumors.

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Materials and methods

Animals. Inbred male BALB/c and C57BL/6 mice, 7–10 weeks old, were purchased from Charles River (Japan) Inc., Tokyo, Japan. They were housed in plastic cages and given food and water freely.

Tumors. The highly metastatic variants of B16 melanoma, B16-F10 and B16-BL6 [2, 6], were kindly provided by Dr. I. J. Fidler. Experimental metastasis occurs after i.v. inoculation of cells of either of these variants into C57BL/6 mice. The metastatic, colonic cancer cell clones, C26NL-17 and C26NL-22, were isolated from a metastatic variant of colonic adenocarcinoma 26 (C26) by Tsuruo et al. [13]. Experimental lung metastasis occurs after i.v. inoculation of C26NL-17, and spontaneous lung metastasis occurs after inoculation of C26NL-22 cells into the front footpads of BALB/c mice. All tumor cells were maintained in vitro in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS). Lewis lung carcinoma (3LL) was maintained by s.c. serial transplantation in C57BL/6 mice. Following aseptic removal, 3LL tumors were minced in Hanks' balanced salt solution (HBSS) and the cell suspension was filtered through a stainless steel mesh. Tumor cells were collected by centrifugation, resuspended in HBSS, counted with a hemocytometer, and adjusted to the desired concentration.

Tumor metastasis and drug administration. CPT-11 was prepared in our institute; the drug's structure is shown in Fig. 1. C26NL-17 cells (5×10^4/mouse) were inoculated i.v. into BALB/c mice (7 mice/group), and CPT-11 was injected i.v. B16-F10 and B16-BL6 cells (5×10^4/mouse) were inoculated i.v. into C57BL/6 mice (7 mice/group) on day 0, and CPT-11 was injected i.v. on days 1–5. The mice were sacrificed on day 21 after tumor cell inoculation and the number of pulmonary metastases were counted.

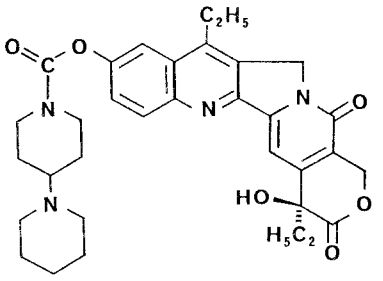

Fig. 1. Structure of CPT-11

C26NL-22 cells (1×10^6/mouse) were inoculated s.c. into the left front footpads of BALB/c mice (7 mice/group) on day 0. CPT-11 was injected i.p. on days 1, 5, and 9, and the forelegs bearing the primary tumors were amputated on day 14. CPT-11 was again injected i.v. on days 17, 20, and 23, and the spontaneous lung metastases were examined on day 30 after tumor inoculation. 3LL cells (5×10^5/mouse) were inoculated s.c. into the left hind footpads of C57BL/6 mice (7 mice/group) on day 0. CPT-11 was injected i.p. or i.v. and the primary tumors were amputated on day 14. CPT-11 was again injected i.v. on days 17, 20, and 23, and spontaneous lung metastases were examined on day 28 after tumor inoculation.

Statistical analysis. The experimental results were analyzed for their statistical significance by Student's *t*-test.

Results

Effect of CPT-11 on experimental metastasis

We first studied the timing of the inoculation of CPT-11 on the formation of lung metastases after i.v. injection of C26NL-17 cells into BALB/c mice. The mice were inoculated i.v. with C26NL-17 cells and CPT-11 was injected i.v. at various times thereafter. The chemotherapy was started on day 1 because we previously found that it was essential to give CPT-11 from the first day after tumor cell inoculation in order for a high antitumor activity to be observed. The number of pulmonary metastases formed in all treated mice was significantly lower than that observed in control animals (Table 1) and was especially effective when the drug was given on days 1–5. Thus, we thereafter adopted this schedule in all experiments concerning the effects of CPT-11 on experimental metastasis. The effect of CPT-11 was found to be dose-dependent, being most effective at 200 mg/kg (Table 2), and was toxic at a dose of more than 300 mg/kg (data not shown). The drug's ability to inhibit lung metastasis by a number of metastatic tumors was subsequently investigated. B16-F10 and B16-BL6 were inoculated i.v. and CPT-11 was injected i.v. on days 1–5. As shown in Tables 3 and 4, it significantly inhibited the formation of metastases by these cells.

Table 1. Antimetastatic effect of CPT-11 against C26NL-17

Group[a]	Total dose[b] (mg/kg)	No. of pulmonary metastases[c]		
		Mean ± SD	Range	Reduction (%)
1	– (control)	158 ± 14	139–183	–
2	200	21 ± 8*	9–32	87
3	200	51 ± 9*	33–66	68
4	200	61 ± 8*	41–70	61
5	200	73 ± 8*	59–83	54

[a] C26NL-17 cells (5×10^4/mouse) were inoculated i.v. into BALB/c mice on day 0
[b] CPT-11 was injected i.v. on days 1–5 (group 2); 1, 3, 5, 7, and 9 (group 3); 1, 4, 7, 10, and 13 (group 4); or 1, 5, and 9 (group 5)
[c] The number of pulmonary nodules was counted on day 21 after tumor inoculation
Statistical significance of difference from group 1: * $P < 0.001$

Table 2. Inhibition of C26NL-17 pulmonary metastasis by CPT-11

Group[a]	Dose (total) (mg/kg)	No. of pulmonary metastases[b]		
		Mean ± SD	Range	Reduction (%)
1	– (control)	131 ± 15	108–151	–
2	200	12 ± 3*	9–18	91
3	100	23 ± 4*	18–29	82
4	50	31 ± 3*	26–37	76
5	25	41 ± 6*	29–50	69

[a] C26NL-17 cells (5×10^4/mouse) were inoculated i.v. into BALB/c mice on day 0 and CPT-11 was injected on days 1–5
[b] The number of pulmonary nodules was counted on day 21 after tumor inoculation
Statistical significance of difference from group 1: * $P < 0.001$

Effect of CPT-11 on spontaneous metastasis

Following the surgical excision of C26NL-22 and 3LL tumors that had grown from cells inoculated into footpads, metastasis was observed to occur. The effects of CPT-11 on spontaneous metastasis in this experimental system were examined. C26NL-22 cells were inoculated s.c. into the left front footpads of BALB/c mice on day 0, and CPT-11 was injected i.p. on days 1, 5, and 9, with the result that the growth of the tumor was significantly inhibited compared with that in control mice (Fig. 2). After surgical excision of the primary tumor on day 14, CPT-11 was again injected i.v. on days 17, 20, and 23 after tumor cell inoculation. As shown in Table 5, CPT-11 effectively inhibited lung metastasis when given before and/or after surgery. Both i.v. and i.p. injections of CPT-11 significantly

Table 3. Inhibition of B16-F10 pulmonary metastasis by CPT-11

Group[a]	Total dose (mg/kg)	No. of pulmonary metastases[b]		
		Mean ± SD	Range	Reduction (%)
1	– (control)	65 ± 17	34–87	–
2	200	16 ± 3**	13–21	75
3	100	34 ± 15*	8–51	48
4	50	38 ± 9*	24–53	42
5	25	54 ± 22	27–97	17

[a] B16-F10 cells (5×10^4/mouse) were inoculated i.v. into C57BL/6 mice on day 0 and CPT-11 was injected i.v. on days 1–5
[b] The number of pulmonary nodules was counted on day 21 after tumor inoculation
Statistical significance of difference from group 1: * $P < 0.05$; ** $P < 0.001$

Table 4. Inhibition of B16-BL6 pulmonary metastasis by CPT-11

Group[a]	Total dose (mg/kg)	No. of pulmonary metastases[b]		
		Mean ± SD	Range	Reduction (%)
1	– (control)	67 ± 23	39 – 101	–
2	200	10 ± 9**	2 – 26	85
3	100	24 ± 12*	11 – 44	64
4	50	23 ± 7*	16 – 35	66
5	25	39 ± 6	30 – 48	42

[a] B16-BL6 cells (5×10^4/mouse) were inoculated i.v. into C57BL/6 mice on day 0 and CPT-11 was injected on days 1 – 5

[b] The number of pulmonary nodules was counted on day 21 after tumor inoculation

Statistical significance of difference from group 1: * $P < 0.05$; ** $P < 0.001$

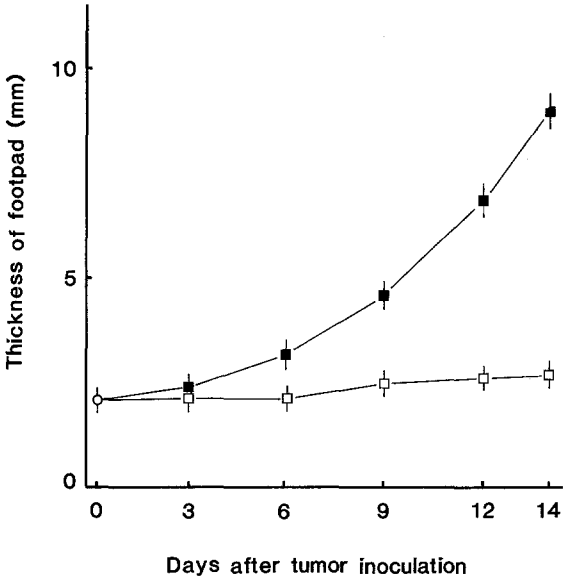

Fig. 2. Effect of CPT-11 on the growth of C26NL-22. C26NL-22 cells (1×10^6/mouse) were inoculated s.c. into the left front footpads of BALB/c mice on day 0. CPT-11 (□) (100 mg/kg) was injected i.p. on days 1, 5, and 9, and tumor growth was monitored for 14 days. The control group (■) was injected with saline

Table 5. Inhibition of spontaneous C26NL-22 lung metastasis by CPT-11

Treatment with CPT-11[a]		No. of pulmonary metastases[b]		
i.p.	i.v.	Mean ± SD	Range	Reduction (%)
–	–	41 ± 11	24 – 55	–
+	–	6 ± 6**	0 – 17	85
–	+	9 ± 4*	4 – 17	78
+	+	4 ± 5**	0 – 11	90

[a] C26NL-22 cells (1×10^6/mouse) were inoculated s.c. into the left front footpad of BALB/c mice on day 0. CPT-11 (100 mg/kg) was injected i.p. on days 1, 5, and 9, and the primary tumors were amputated on day 14. CPT-11 (100 mg/kg) was injected i.v. on days 17, 20, and 23

[b] Spontaneous lung metastases were examined on day 30 after tumor inoculation

Statistical significance of difference from nontreated control: * $P < 0.01$; ** $P < 0.001$

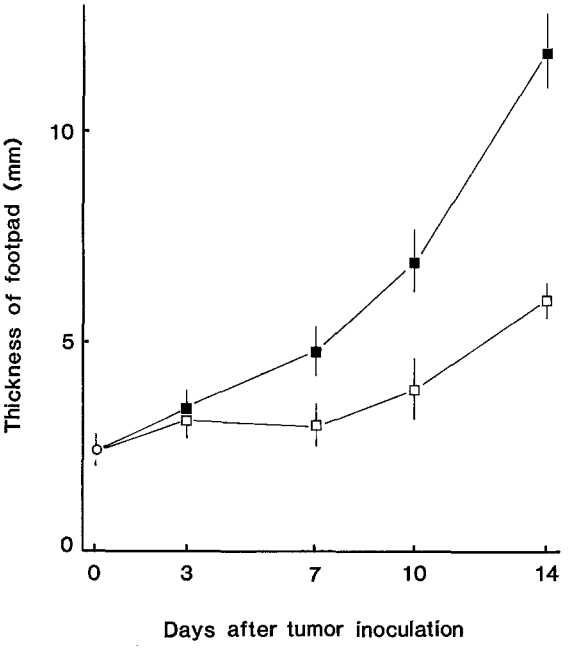

Fig. 3. Effect of CPT-11 on the growth of 3LL. 3LL cells (5×10^5/mouse) were inoculated s.c. into the left hind footpads of C57BL/6 mice on day 0. CPT-11 (□) (100 mg/kg) was injected i.p. on days 1, 5, and 9, and tumor growth was monitored for 14 days. The control group (■) was injected with saline

inhibited lung metastasis; metastasis-free mice were observed. Furthermore, both i.p. and i.v. injections of CPT-11 effectively inhibited the growth of 3LL primary tumors inoculated s.c. into the left hind footpads of C57BL/6 mice (Figs. 3 and 4). After surgical excision of the primary tumor, subsequent i.v. injection of CPT-11 on days 17, 20,

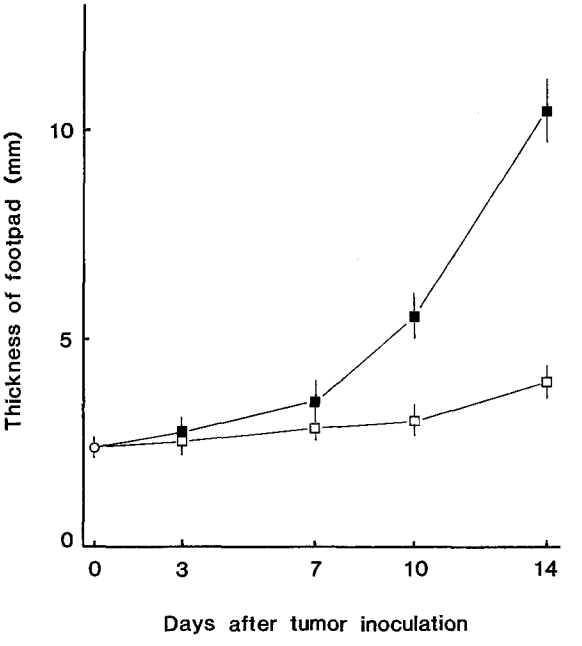

Fig. 4. Antitumor effect of i.v. injection of CPT-11 against 3LL cells. 3LL cells (5×10^5/mouse) were inoculated s.c. into the left hind footpads of C57BL/6 mice on day 0. CPT-11 (□) (100 mg/kg) was injected i.v. on days 1 – 5, and tumor growth was monitored for 14 days. The control group (■) was injected with saline

Table 6. Inhibition of spontaneous 3LL lung metastasis by CPT-11

Treatment with CPT-11[a]		No. of pulmonary metastases[b]		
i.p.	i.v.	Mean ± SD	Range	Reduction (%)
−	−	56 ± 25	20–89	−
+	−	10 ± 6*	2–17	82
−	+	10 ± 6*	2–19	82
+	+	3 ± 2**	0–6	95

[a] 3LL cells (5×10^5/mouse) were inoculated s.c. into the left hind footpad of C57BL/6 mice on day 0. CPT-11 (100 mg/kg) was injected i.p. on days 1, 5, and 9, and the primary tumors were amputated on day 14. CPT-11 (100 mg/kg) was injected i.v. on days 17, 20, and 23

[b] Spontaneous lung metastases were examined on day 28 after tumor inoculation

Statistical significance of difference from nontreated control: * $P < 0.01$; ** $P < 0.001$

Table 7. Antimetastatic effect of CPT-11 against 3LL

Treatment with CPT-11[a]		No. of pulmonary metastases[b]		
i.v.	i.v.	Mean ± SD	Range	Reduction (%)
−	−	81 ± 7	70–92	−
+	−	3 ± 4*	0–11	96
−	+	10 ± 8*	0–25	88
+	+	2 ± 3*	0–10	98

[a] 3LL cells (5×10^5/mouse) were inoculated s.c. into the hind footpad of C57BL/6 mice on day 0. CPT-11 (100 mg/kg) was injected i.v. on days 1, 5, and the primary tumors were amputated on day 14. CPT-11 (100 mg/kg) was injected i.v. on days 17, 20, and 23

[b] Spontaneous lung metastases were examined on day 28 after tumor cell inoculation

Statistical significance of difference from nontreated control: * $P < 0.001$

and 23 reduced lung metastasis. Combined i.p. and i.v. injections were effective in the prevention of lung metastasis (Table 6), and i.v. injection of CPT-11 before and after surgical excision of the primary tumor also markedly inhibited lung metastasis (Table 7).

Discussion

Camptothecin shows strong antitumor activity, but also high toxicity. Thus, there have been many attempts to synthesize derivatives with both a high potential for inhibiting tumor growth and a reduced toxicity [14, 16–18]. 7-Ethyl-camptothecin (CPT-11), one of these derivatives, has been found to inhibit tumor growth and to induce prolongation of the survival times for tumor-bearing mice [8]. Yokokura et al. have found that CPT-11 is water-soluble, can more effectively inhibit tumor growth, and exhibits lower toxicity than camptothecin itself [20, 21].

We demonstrated in the present study that i.v. injection of CPT-11 effectively inhibited experimental metastasis of the highly metastatic tumors C26NL-17, B16-F10, and B16-BL6, and that both i.v. and i.p. injections of CPT-11 inhibited not only the growth of s.c. transplanted tumors but also spontaneous lung metastasis by C26NL-22 and 3LL tumors. The i.v. injection of CPT-11 into C26NL-17-, B16-F10-, and B16-BL6-bearing mice markedly inhibited the formation of lung metastases, the maximal reduction being greater than 90% compared with control mice. Furthermore, i.p. injection of CPT-11 significantly inhibited the growth of C26NL-22 inoculated s.c. into the front footpads of mice. It was also observed that both i.p. and i.v. injections of CPT-11 inhibited the growth of 3LL tumors inoculated s.c. into the hind footpads. Combinations of i.p. and i.v. and of i.v. and i.v. injections before and/or after surgical excision of the primary tumors also inhibited lung metastasis. Thus, it was concluded that both i.p. and i.v. injections of CPT-11 inhibited tumor-induced thickening of mouse footpads, subsequently resulting in inhibition of lung metastasis. These data suggest that CPT-11 effectively inhibits not only primary tumor growth but also the formation of lung metastases by several highly metastatic murine tumors, and it is thus expected to be useful in clinical trials. Hereafter, further investigation will be required to clarify the detailed mechanism involved in the inhibition of cancer metastases by CPT-11.

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