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ORIGINAL ARTICLE

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Potent and broad antitumor effects of DX-8951f, a water-soluble camptothecin derivative, against various human tumors xenografted in nude mice

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Abstract *Purpose*: We have previously reported that DX-8951f, a water-soluble and nonprodrug camptothecin (CPT) derivative, exhibits both high in vitro potency against a series of 32 malignant cell lines and significant topoisomerase I inhibition. The purpose of this study was to evaluate the therapeutic efficacy of DX-8951f against human tumor xenografts in nude mice and to compare its activity with those of CPT-11 and other current CPT derivatives. *Methods*: The antitumor activity of DX-8951f against xenografts of several different types of human tumors was determined in nude mice using a schedule in which DX-8951f was administered intravenously every 4th day for a total of four injections. *Results*: Against both gastric adenocarcinoma SC-6 and its CPT-11-resistant variant, SC-6/CPT-11, DX-8951f demonstrated superior antitumor activity and antitumor activity over a broader range of doses than did CPT-11, SK&F104864 (hycamtin, topotecan) and GG-211 (GI147211). DX-8951f at 75 mg/kg was effective (growth inhibition rate IR $\geqq$ 58%) against 15 of 16 lines of human cancers examined (6 colon cancers, 5 lung cancers, 2 breast cancers, 1 renal cancer and the above 2 gastric cancers), and exhibited excellent antitumor activity (IR $\geqq$ 80%) against 14 of these lines. CPT-11 exhibited antitumor activity with IR values of 58% and higher against 11 lines and IR values of 80% and higher against only eight of the same 16 human tumors. DX-8951f was effective in inhibiting the growth of an SN-38-resistant tumor and some P-glycoprotein-expressing tumors, but CPT-11 was not. *Conclusions*: DX-8951f exhibited potent antitumor activity against various types of human tumor xenografts. Its in vivo antitumor effects were superior to those of current camptothecin analogs against certain tumors.

Key words DX-8951f · CPT-11 · Camptothecin derivatives · Human tumor xenografts

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Introduction

CPT-11, a DNA topoisomerase I inhibitor, has a mechanism of action different from other currently available therapeutic agents [13]. It exhibits antitumor activity against some human tumor xenografts in nude mice when administered intravenously (i.v.) as a single dose or when administered intermittently. CPT-11 is effective against human colon cancers Co-4 [14] and H-110 [5], lung cancers QG56 [14] and H-74 [5], and against breast cancer MX-1 [14]. Evaluation of phase II trials with CPT-11 has revealed a high response rate among small-cell lung cancers [17, 20], non-small-cell lung cancers [6,18], cervical cancers [23], ovarian cancers [22] and colorectal cancers [21]. The response rate to CPT-11 is 25% in colorectal cancer patients with prior radiotherapy or chemotherapy [21]. These findings demonstrate that the antitumor activity of CPT-11 observed in the preclinical studies also could be obtained clinically, and suggest that results of studies using human tumor xenografts in nude mice can be used to predict the clinical efficacy of future antitumor agents.

We previously reported that DX-8951f, a new camptothecin derivative, exhibits significant topoisomerase I inhibition and potent antitumor activity in vitro against human tumors derived from a variety of tissues. These effects of DX-8951f are greater than those of CPT-11 and SK&F104864 [19]. In the present study, we examined the therapeutic efficacy of DX-8951f against a variety of human tumor xenografts in nude mice and compared its activity with those of CPT-11 and other current camptothecin derivatives.

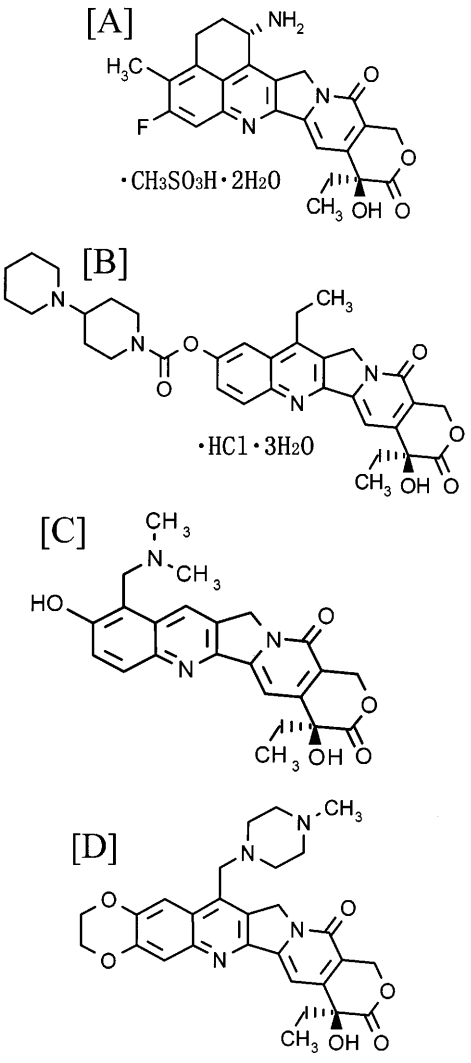

Fig. 1A–D Chemical structures of DX-8951f (**A**), CPT-11 (**B**), SK&F104864 (**C**) and GG-211 (**D**)

Materials and methods

Compounds

DX-8951f (Fig. 1A), (1*S*,9*S*)-1-amino-9-ethyl-5-fluoro-2,3-dihydro-9-hydroxy-4-methyl-1*H*,12*H*-benzo [*de*] pyrano [3′,4′:6,7] indolizino [1,2-*b*] quinoline-10,13 (9*H*,15*H*)-dione monomethanesulfonate dihydrate, was synthesized entirely in our laboratory. CPT-11 (Fig. 1B) was provided by Yakult Honsha Co. (Tokyo, Japan). SK&F104864 (Fig. 1C) and GG-211 (Fig. 1D) were synthesized as described previously [11,15,16]. Prior to use, DX-8951f, CPT-11, SK&F104864 and GG-211 were dissolved in pyrogen-free distilled water (Otsuka Pharmaceutical Co., Tokyo, Japan) and diluted using the same water. Dose levels of the above compounds are expressed as those of the anhydrous free base. Vincristine (VCR, Shionogi Co., Osaka, Japan) was dissolved and diluted with pyrogen-free physiological saline (Otsuka Pharmaceutical Co.).

Animals

Male 6-week-old BALB/c-nu/nu nude mice were purchased from Japan SLC (Shizuoka, Japan). They were housed in an exclusively experimental room under specific pathogen-free conditions, and food sterilized by γ irradiation (Oriental Yeast Co., Tokyo, Japan) and water treated with hypochlorite were available ad libitum.

Tumors

The human tumor lines examined are listed in Table 1. SC-6 and MX-1 were supplied by the Cancer Chemotherapy Center, Japanese Foundation for Cancer Research, Tokyo, Japan. Co-3 and Co-4 were kindly supplied by Dr. T. Kubota, School of Medicine, Keio University, Tokyo, Japan. SW620, SK-CO-1, DLD-1, WiDr, Du4475 and A-498 were obtained from the American Type Culture Collection, Rockville, Md. PC-12 was supplied by Toray Co., Tokyo, Japan. PC-6 and PC-14 were obtained from Immuno-Biological Laboratories, Gunma, Japan. SC-6/CPT-11, a CPT-11-resistant variant, was obtained by i.v. administration to SC-6-bearing nude mice of CPT-11 at 60 mg/kg once or twice per week for 2 months. PC-6/VCR29-9 and PC-6/SN2-5S were isolated from PC-6 by stepwise selection with increasing VCR and SN-38 concentrations, respectively. DLD-1, PC-12 and PC-6/VCR29-9 were confirmed to overexpress P-glycoprotein [19]. SC-6, SC-6/CPT-11, Co-3, Co-4 and MX-1 were maintained by sequential subcutaneous (s.c.) transplantation into nude mice. Other lines were cultured in vitro in RPMI-1640 medium (Gibco-BRL, N.Y.) supplemented with 10% fetal bovine serum (FBS; Hyclone Laboratories, Utah, or Bocknek, Canada), and then inoculated s.c. into nude mice and maintained as solid tumors in the manner described above.

Evaluation of antitumor effects

Various human tumors maintained in nude mice were excised and cut into pieces approximately 2 to 3 mm in diameter in endotoxin-free (less than 50 pg/ml) Hanks' balanced salt solution (HBSS, Gibco-BRL). A piece of tumor was transplanted s.c. into the right flank of nude mice using a trocar. When the mean estimated tumor weight (ETW) reached 100 to 200 mg between days 10 and 40 after tumor transplantation, the mice were randomly divided into experimental groups (five or six mice per group) and were treated i.v. with a test compound every 4th day for a total of four injections (q4d × 4), every 4th day for a total of three injections (q4d × 3), every 7th day for a total of three injections (q7d × 3) or daily for 5 days (qd × 5). The ETW was calculated using the formula $ETW = L \times W^2/2$, where L and W represent the length and the width of the tumor mass, respectively. After the first administration on day 0, the ETW and body weight of the mice were measured two to five times per week for 22 or 28 days. The tumor masses were then excised and weighed. The growth inhibition rate (IR) on the basis of tumor weight was calculated using the formula $IR = (1 - TWt/TWc) \times 100$ (%), where TWt represents the mean of tumor weight of a treated group and TWc represents that of the control group.

The significance of differences in tumor weights between test and control groups was analyzed using Dunnett's test or the Tukey-Kramer test. When the IR was 58% and higher, the drug was evaluated as effective [4]. Conversely, when the IR was less than 58%, the drug was evaluated as ineffective even if statistical significance was shown. Furthermore, to evaluate the intensity of the side effects of compounds, the rate of body weight loss (BWL) and D/U were utilized as parameters of toxicity. BWL was calculated using the formula $BWL = (1 - BWn/BWs) \times 100$ (%), where BWn and BWs represent the mean body weights of mice on day *n* and on the day of initial administration, respectively. The maximum value of BWL was designated as BWLmax, and BWLmax less than zero indicates no body weight loss. D/U indicates the ratio of the number of mice that died of toxic effects to the number of mice used.

Table 1 Human cell lines used in the experiments

Gastric cancer	SC-6[a]	Poorly differentiated adenocarcinoma
	SC-6/CPT-11[b]	CPT-11-selected variant of SC-6
Colon cancer	Co-3[c]	Well-differentiated adenocarcinoma
	Co-4[c]	Poorly differentiated adenocarcinoma
	SW620[d]	Adenocarcinoma
	SK-CO-1[d]	Adenocarcinoma
	DLD-1[d,g]	Adenocarcinoma
	WiDr[d]	Adenocarcinoma
Lung cancer	PC-12[e,g]	Differentiated adenocarcinoma
	PC-14[f]	Poorly differentiated adenocarcinoma
	PC-6[f]	Oat-cell carcinoma
	PC-6/VCR29-9[b,g]	VCR-selected variant of PC-6
	PC-6/SN2-5S[b]	SN-38-selected variant of PC-6
Breast cancer	Du4475[d]	Carcinoma
	MX-1[a]	Medullary tubular carcinoma
Renal cancer	A-498[d]	Carcinoma

[a] Supplied by Cancer Chemotherapy Center, Japanese Foundation for Cancer Research, Tokyo
[b] Drug-resistant cell lines established in our laboratory
[c] Supplied by Dr. T. Kubota, Keio University, School of Medicine, Tokyo
[d] Obtained from the American Type Culture Collection, Rockville, Md
[e] Supplied by Toray Co., Ltd., Tokyo
[f] Obtained from Immuno-Biological Laboratories, Gunma, Japan
[g] P-glycoprotein overexpression

Results

Antitumor effects of DX-8951f in various schedules against human SC-6 gastric cancer xenografts

To determine the schedule of administration used in further experiments, the therapeutic efficacies of DX-8951f when administered every 4th day for a total of four injections (q4d × 4), every 4th day for a total of three injections (q4d × 3) or every 7th day for a total of three injections (q7d × 3) against SC-6 xenografts were examined (Table 2). DX-8951f exhibited strong antitumor effects with IR values of 77% and higher at total doses of 25 and 50 mg/kg on all schedules. In a comparison of growth curves at 50 mg/kg (Fig. 2), it was tendentiously observed that the q4d × 4 schedule more readily caused the regressive effects of DX-8951f compared with the q7d × 3, and a schedule of q4d × 3 more frequently permitted regrowth of tumors than did the q4d × 4 schedule. Based on these findings, the q4d × 4 schedule was chosen as a schedule for intermittent application of DX-8951f, and the antitumor effects of the compound on the schedule against human tumor xenografts in nude mice were examined.

Antitumor effects against gastric cancer xenografts

The results are shown in Table 3. The antitumor effects of various camptothecin derivatives (DX-8951f at a total dose of 25 mg/kg, SK&F104864 at a total dose of

Table 2 Antitumor effects of DX-8951f in various schedules against human SC-6 gastric cancer xenografts in nude mice. A piece of SC-6 tumor block was inoculated into the right flank of BALB/c-nu/nu mice (day 0). Mice received multiple i.v. administrations on q4d × 4, q4d × 3 or q7d × 3 schedules from day 15 (experiment 1) or 14 (experiment 2). Tumor weight was assessed on day 43 (experiment 1) or 42 (experiment 2). Dose levels of DX-8951f are expressed as those of the anhydrous free base

Total dose (mg/kg)	Schedule	IR (%)	BWLmax[a] %	BWLmax[a] Day	D/U[b]
Experiment 1[c]					
50	q4d × 4	97	9.9	21	0/6
	q4d × 3	92	12.5	27	0/6
	q7d × 3	93	6.7	32	0/6
Experiment 2[d]					
25	q4d × 4	94	4.3	23	0/6
	q4d × 3	88	4.9	19	0/6
	q7d × 3	77	1.6	15	0/6
6.25	q4d × 4	58	2.8	15	0/6
	q4d × 3	45	< 0		0/6
	q7d × 3	50	< 0		0/6

** $P < 0.01$, * $P < 0.05$, Tukey-Kramer test
[a] Maximum rate of body weight loss (< 0 indicates no body weight loss)
[b] Number of mice that died of toxicity/number of mice used
[c] Tumor weight of control group: 4.797 ± 0.456 g
[d] Tumor weight of control group: 3.815 ± 0.398 g

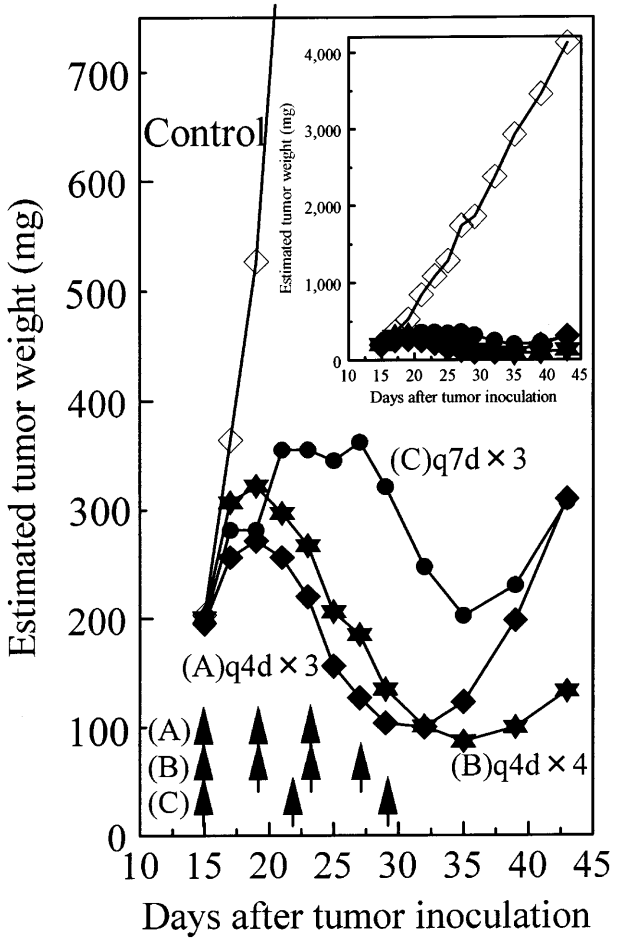

Fig. 2 Inhibitory effects of DX-8951f at 50 mg/kg as total dose in various schedules against human SC-6 gastric cancer xenografts in nude mice. Dose levels of DX-8951f are expressed as those of the anhydrous free base. The changes in estimated tumor weight observed in experiment 1 of Table 2 were plotted. *Arrows* indicate days of treatment on each schedule

30 mg/kg and GG-211 at a total dose of 25 mg/kg) against SC-6 xenografts were examined twice (experiments 1 and 2), and nearly equal IR values were obtained in the two experiments. The dose-effect curves, which represent the relationship between IR values and total doses of each compound, are shown in Fig. 3. DX-8951f exhibited a strong antitumor effect on SC-6, with IR values of 94% and higher at total doses of 75, 50 and 25 mg/kg without deaths due to toxicity, and with an IR value of 58% even at a total dose of 6.25 mg/kg, one-twelfth the highest dose. CPT-11 also exhibited an antitumor effect with IR values over 58% at all doses examined: an IR value of 94% was obtained at the highest total dose of 320 mg/kg, but the IR was reduced to 68% at a total dose of 160 mg/kg, half the highest dose. SK&F104864 at a total dose of 45 mg/kg showed an IR value of 81%, but resulted in deaths from toxicity (two of six mice). SK&F104864 failed to show an antitumor effect even at the highest nonlethal dose of 30 mg/kg. GG-211 was highly toxic at total doses of 75 and 50 mg/kg. GG-211 exhibited an antitumor effect with an IR value over 90% at a total dose of 25 mg/kg without deaths from toxicity, but was not effective at a total dose of 6.25 mg/kg (IR 39%), one-fourth the maximum tolerated dose (MTD).

Against SC-6/CPT-11, CPT-11 at a total dose of 320 mg/kg showed an IR value of 65%, but this value was low compared with its IR value of 94% against SC-6. This finding confirmed that SC-6/CPT-11 is resistant to CPT-11. DX-8951f showed a strong antitumor effect with IR values of 94% and higher at total doses of 75 and 50 mg/kg against both SC-6/CPT-11 and the parent tumor. SK&F104864 was not effective against SC-6/CPT-11 at total doses of 20 and 30 mg/kg, at which no mice died of toxicity. GG-211 at a total dose of 25 mg/kg resulted in an IR value of 69% against SC-6/CPT-11, but this effect was weaker than that against SC-6.

Antitumor effects against colon cancer xenografts

The results are shown in Table 4. DX-8951f at total doses of 75 and 50 mg/kg showed antitumor effects with IR values of about 80% against Co-3, Co-4 and DLD-1. Against Co-3 and Co-4 in particular, the compound exhibited antitumor effects with IR values of about 60% even at a total dose of 25 mg/kg. Furthermore, DX-8951f showed strong antitumor effects with IR values of about 90% against SK-CO-1 and WiDr at all doses examined and IR values of 96 to 100% at doses of 25 to 75 mg/kg against SW620. Thus, against all the above colon cancer lines, DX-8951f resulted in IR values of 80% and higher at 75 mg/kg and IR values above 58% at 50 mg/kg or, in selected tumors, at 25 mg/kg.

CPT-11 showed a strong antitumor effect against Co-3, Co-4 and SW620 with IR values of 90% and higher, and against SK-CO-1 with IR values of 62% and higher at total doses of 160 mg/kg or more. However, CPT-11 showed no or only a slight antitumor effect with IR values of 48% and 59% against WiDr and DLD-1 even at the highest dose of 320 mg/kg. The effects against these two tumor lines were weaker than those of DX-8951f.

Antitumor effects against lung cancer xenografts

The results for PC-12 and PC-14 are shown in Table 5. DX-8951f showed antitumor effects against these lung cancers with IR values of 91% and higher at total doses of 75 mg/kg and IR values of 60% to 70% at 50 mg/kg, without marked body weight loss. CPT-11 was not effective against PC-12. CPT-11 showed antitumor effects against PC-14 with IR values of 83% and 59% at total doses of 320 and 240 mg/kg. This effect was somewhat inferior to that of DX-8951f.

The results for PC-6 and its resistant variants are shown in Table 6, and the dose-effect curves for

Table 3 Antitumor effects of various camptothecin derivatives against human gastric cancer xenografts in nude mice. A piece of tumor block was implanted into the right flank of BALB/c-nu/nu mice (day 0). The mice were randomized and divided into several groups (six mice per group) when the mean estimated tumor weight reached 82 to 178 mg (SC-6 experiment 1, day 10; SC-6 experiment 2, day 14; SC-6/CPT-11, day 22). Groups of mice received i.v. injections of DX-8951f, CPT-11, SK&F104864 or GG-211 every 4th day for a total of four injections (q4d × 4). Tumor weight was assessed on the 28th day after the first administration. The tumor weights of the control groups were as follows: SC-6 experiment 1, 3.872 ± 0.335 g; experiment 2, 3.815 ± 0.398 g; SC-6/CPT-11, 1.122 ± 0.389 g. Dose levels of compounds are expressed as those of the anhydrous free base (*ND* not done)

Compound	Total dose[a] (mg/kg)	SC-6								SC-6/CPT-11			
		Experiment 1				Experiment 2							
		IR	BWLmax[b]		D/U[c]	IR	BWLmax[b]		D/U[c]	IR	BWLmax[b]		D/U[c]
		(%)	(%)	Day		(%)	%	Day		(%)	%	Day	
Control	0	0	< 0		0/6	0	< 0		0/6	0	< 0		0/6
DX-8951f	75 (18.75 × 4)	98***	3.9	16	0/6	ND				96**	22.1	37	0/6
	50 (12.5 × 4)	98***	3.1	16	0/6	ND				94**	6.9	31	0/6
	25 (6.25 × 4)	94***	2.6	15	0/6	94***	4.3	23	0/6	67*	1.2	23	0/6
	12.5 (3.125 × 4)	ND				ND				57	1.4	23	0/6
	6.25 (1.5625 × 4)	ND				58***	2.8	15	0/6	39	4.8	23	0/6
CPT-11	320 (80 × 4)	94***	3.9	12	0/6	ND				65	1.5	31	0/6
	240 (60 × 4)	77***	0.8	11	0/6	ND				59	1.2	28	0/6
	160 (40 × 4)	68***	< 0		0/6	ND				56	0.3	28	0/6
SK&F104864	45 (11.25 × 4)	ND				81***	9.6	19	2/6	65	22.5	31	3/6
	30 (7.5 × 4)	53***	< 0		0/6	53***	1.0	18	0/6	41	7.2	31	0/6
	20 (5 × 4)	28*	< 0		0/6	ND				28	0.4	24	0/6
	10 (2.5 × 4)	32*	< 0		0/6	ND				ND			
GG-211	75 (18.75 × 4)	Toxic	32.2	18	6/6	ND				ND			
	50 (12.5 × 4)	99***	30.9	22	5/6	ND				ND			
	25 (6.25 × 4)	95***	2.1	16	0/6	91***	3.9	19	0/6	69	17.7	38	0/6
	12.5 (3.125 × 4)	ND				ND				18	2.7	28	0/6
	6.25 (1.5625 × 4)	ND				36**	1.4	15	0/6	7	< 0		0/6

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; versus control by Dunnett's test

[a] Compounds were administered four times at 4-day intervals

[b] Maximum rate of body weight loss (< 0 indicates no body weight loss)

[c] Number of mice that died of toxicity/number of mice used

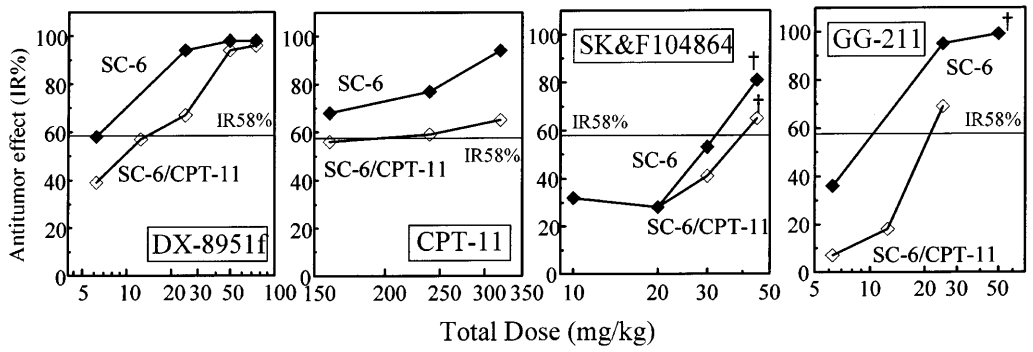

Fig. 3 Dose-effect curves for DX-8951f, CPT-11, SK&F104864 and GG-211 against human gastric cancers SC-6 (◆) and SC-6/CPT-11 (◇) in nude mice. Dose levels of the compounds are expressed as those of the anhydrous free base. Values of IR% are plotted against the total dose of each derivative. Data were obtained from Table 3. † indicates that some mice died of toxic effects

compounds against these tumor lines are shown in Fig. 4. DX-8951f exhibited antitumor effects against parental PC-6 at total doses of 25 and 50 mg/kg with IR values of 74% and 98%, respectively, without deaths from toxicity. It also showed strong antitumor effects against PC-6/SN2-5S and PC-6/VCR29-9 with IR values of 79% and 93% at a total dose of 75 mg/kg. CPT-11 showed a moderate antitumor effect (IR 63%) against PC-6 only at the highest dose of 320 mg/kg. CPT-11 was not effective against the resistant variants PC-6/SN2-5S and PC-6/VCR29-9. On the other hand, VCR at total doses of 3 and 2.25 mg/kg showed antitumor effects against PC-6 and PC-6/SN2-5S with IR values of about 70%. VCR was not effective against PC-6/VCR29-9.

Antitumor effects against breast cancer xenografts

The results are shown in Table 7. DX-8951f at total doses of 75 and 50 mg/kg, as well as CPT-11 at 320 and 160 mg/kg, showed strong antitumor effects against MX-1 and Du4475. Against MX-1, DX-8951f and CPT-11 caused complete regression of tumor masses.

Antitumor effects against renal cancer xenografts

The results are shown in Table 8. DX-8951f at total doses of 50 and 75 mg/kg exhibited no antitumor activity against A-498. CPT-11 also showed no antitumor effect against A-498.

Comparison of the efficacy of DX-8951f and CPT-11

The efficacies of DX-8951f and CPT-11 against human tumor xenografts of 16 lines examined are summarized in Table 9. The number of tumors against which DX-8951f or CPT-11 exhibited IR values of 80% and higher or IR values of 58% and higher at the MTD (75 mg/kg and 320 mg/kg, respectively) are shown as indexes of antitumor effects. Response rates are given in parentheses. DX-8951f exhibited antitumor effects with IR values of 58% and higher against 15 lines (94%) and with IR values of 80% and higher against 10 lines (88%). On the other hand, CPT-11 exhibited antitumor effects with IR values of 58% and higher against 11 lines (69%) and with IR values of 80% and higher against 8 lines (50%).

Discussion

We have previously reported that DX-8951f exhibits significant topoisomerase I inhibition and potent antitumor activity in vitro against human tumors derived from a variety of tissues, and that these effects of DX-8951f are greater than those of CPT-11 and SK&F104864 [19]. In the present study, we examined the therapeutic efficacy of DX-8951f against a variety of human tumor xenografts in nude mice. The schedule of administration was established as intermittent application (q4d × 4) based on experimental results (Table 2) and reports that CPT-11 [14], SK&F104864 [9] and GG-211 [3] by intermittent applications exhibit significant antitumor effects against human tumor xenografts.

First, the antitumor activity of DX-8951f against SC-6 gastric cancer xenografts was compared with those of CPT-11, SK&F104864 and GG-211 (Table 3). DX-8951f exhibited antitumor effects with IR values of 94% and higher at total doses of 75 (MTD), 50 and 25 mg/kg and with IR value of 58% even at one-twelfth the MTD, 6.25 mg/kg. When the therapeutic ratio [the ratio of MTD to minimum effective dose, MED (MTD/MED)] was compared, the ratio for DX-8951f (MTD/MED 12) was clearly higher than that of other camptothecin derivatives (MTD/MED less than 2). In addition, DX-8951f exhibited strong antitumor effects at total doses of 75 and 50 mg/kg against SC-6/CPT-11, as well as against the parent tumor. However, the efficacy of the other derivatives was less than this against SC-6/CPT-11. These findings indicate that the antitumor activity and the effective dose ranges of DX-8951f are superior to those of the current camptothecin derivatives against human tumor xenografts.

Table 4 Antitumor effects of DX-8951f and CPT-11 against human colon cancer xenografts in nude mice. A piece of tumor block was implanted into the right flank of BALB/c-nu/nu mice (day 0). The mice were randomized and divided into several groups (six mice per group) when the mean estimated tumor weight reached 100 to 200 mg (Co-3 day 15, Co-4 day 18, SW620 day 11, SK-CO-1 day 41, DLD-1 day 14, WiDr day 21). Groups of mice received i.v. injections of DX-8951f or CPT-11 every 4th day for a total of four injections (q4d × 4). Tumor weight was assessed on the 22nd day (Co-3) or the 28th day (Co-4, SW620, SK-CO-1, DLD-1, WiDr) after the first administration. The tumor weights of control groups were as follows: Co-3 1.504 ± 0.205 g, Co-4 2.386 ± 0.404 g, SW620 4.222 ± 0.762 g, SK-CO-1 0.508 ± 0.097 g, DLD-1 1.279 ± 0.127 g, WiDr 1.052 ± 0.138 g. The dose levels of the compounds are expressed as those of the anhydrous free base (*ND* not done)

Compound	Total dose[a] (mg/kg)	Co-3				Co-4				SW620			
		IR	BWLmax[b]		D/U[c]	IR	BWLmax[b]		D/U[c]	IR	BWLmax[b]		D/U[c]
		(%)	%	Day		(%)	%	Day		(%)	%	Day	
Control	0	0	9.0	35	0/4[d]	0	11.2	40	0/6	0	< 0		0/6
DX-8951f	75 (18.75 × 4)	80***	15.8	28	0/6	85***	15.4	34	0/6	100***	2.8	20	0/6
	50 (12.5 × 4)	78***	11.8	28	0/6	76***	11.9	32	0/6	99***	0.6	20	0/6
	25 (6.25 × 4)	59***	13.4	32	0/6	61***	13.3	32	0/6	96***	1.8	16	0/6
	12.5 (3.125 × 4)	47**	13.9	35	0/6	51**	15.5	40	0/6	ND			
	6.25 (1.5625 × 4)	43**	9.6	35	0/6	57***	18.4	40	0/6	ND			
CPT-11	320 (80 × 4)	95***	12.0	28	0/6	97***	9.2	27	0/6	99***	1.8	27	0/6
	240 (60 × 4)	89***	12.7	28	0/6	ND				99***	< 0		0/6
	160 (40 × 4)	91***	8.3	28	0/6	78***	10.9	34	0/6	94***	0.2	27	0/6
	80 (20 × 4)	86***	7.3	28	0/6	63***	17.3	40	0/6	ND			

Compound	Total dose[a] (mg/kg)	SK-CO-1				DLD-1				WiDr			
		IR	BWLmax[b]		D/U[c]	IR	BWLmax[b]		D/U[c]	IR	BWLmax[b]		D/U[c]
		(%)	%	Day		(%)	%	Day		(%)	%	Day	
Control	0	0	7.2	69	0/6	0	< 0		0/6	0	< 0		0/6
DX-8951f	75 (18.75 × 4)	88***	25.7	57	0/6	86***	5.6	28	0/6	90***	2.0	30	0/6
	50 (12.5 × 4)	89***	16.1	57	0/6	81***	2.2	28	0/6	93***	< 0		0/6
	25 (6.25 × 4)	88***	14.1	55	0/6	ND				ND			
CPT-11	320 (80 × 4)	85***	13.2	55	0/6	59**	1.2	26	0/6	48*	3.5	35	0/6
	240 (60 × 4)	76***	10.6	55	0/6	42*	1.9	26	0/6	36	< 0		0/6
	160 (40 × 4)	62**	9.2	55	0/6	ND				ND			

* $P < 0.05$, ** $P < 0.001$, *** $P < 0.001$; versus control by Dunnett's test

[a] Compounds were administered 4 times at 4-day intervals

[b] Maximum rate of body weight loss (< 0 indicates no body weight loss)

[c] Number of mice that died of toxicity/number of mice used

[d] Two mice in the control group were eliminated because they died of tumor on days 32 and 35

Table 5 Antitumor effects of DX-8951f and CPT-11 against human lung cancer xenografts in nude mice. A piece of tumor block was implanted into the right flank of BALB/c-nu/nu mice (day 0). The mice were randomized and divided into several groups (five or six mice per group) when the mean estimated tumor weight reached 108 to 202 mg (PC-12 day 12, PC-14 day 16). Groups of mice received i.v. injections of DX-8951f or CPT-11 every 4th day for a total of four injections (q4d × 4). Tumor weight was assessed on the 22nd day (PC-12) or the 28th day (PC-14) after the first administration. The tumor weights of the control groups were as follows: PC-12 5.027 ± 0.975 g, PC-14 6.586 ± 0.343 g. The dose levels of the compounds are expressed as those of the anhydrous free base

Compound	Total dose[a] (mg/kg)	PC-12				PC-14			
		IR	BWLmax[b]		D/U[c]	IR	BWLmax[b]		D/U[c]
		(%)	%	Day		(%)	%	Day	
Control	0	0	< 0		0/6	0	< 0		0/5
DX-8951f	75 (18.75 × 4)	91***	< 0		0/6	95***	2.6	25	0/5
	50 (12.5 × 4)	63**	< 0		0/6	71***	3.5	21	0/5
CPT-11	320 (80 × 4)	12	< 0		0/6	83***	< 0		0/5
	240 (60 × 4)	19	< 0		0/6	59***	< 0		0/5

** $P < 0.01$, *** $P < 0.001$; versus control by Dunnett's test
[a] Compounds were administered four times at 4-day intervals
[b] Maximum rate of body weight loss (< 0 indicates no body weight loss)
[c] Number of mice that died of toxicity/number of mice used

Table 6 Antitumor effects of DX-8951f, CPT-11 and VCR against human lung cancer xenografts in nude mice. A piece of tumor block was implanted into the right flank of BALB/c-nu/nu mice (day 0). The mice were randomized and divided into several groups (six mice per group) when the mean estimated tumor weight reached 124 to 163 mg (PC-6 day 21, PC-6/SN2-5S day 22, PC-6/VCR29-9 day 36). Groups of mice received i.v. injections of DX-8951f or CPT-11 every 4th day for a total of four injections (q4d × 4) or VCR daily for 5 days (qd × 5). Tumor weight was assessed on the 28th day after the first administration. The tumor weights of the control groups were as follows: PC-6 2.455 ± 0.267 g, PC-6/SN2-5S 1.822 ± 0.259 g, PC-6/VCR29-9 1.870 ± 0.377 g. The dose levels of the camptothecin derivatives are expressed as those of the anhydrous free base (*ND* not done)

Compound	Total dose[a] (mg/kg)	PC-6				PC-6/SN2-5S				PC-6/VCR29-9			
		IR	BWLmax[b]		D/U[c]	IR	BWLmax[b]		D/U[c]	IR	BWLmax[b]		D/U[c]
		(%)	%	Day		(%)	%	Day		(%)	%	Day	
Control	0	0	< 0		0/6	0	< 0		0/6	0	< 0		0/6
DX-8951f	75 (18.75 × 4)	99	28.8	30	4/6	79***	14.3	37	0/6	93**	15.2	52	0/6
	50 (12.5 × 4)	98***	17.9	35	0/6	65***	14.4	37	0/6	ND			
	25 (6.25 × 4)	89***	4.3	35	0/6	51**	< 0		0/6	ND			
	12.5 (3.125 × 4)	74***	< 0		0/6	45**	< 0		0/6	ND			
	6.25 (1.5625 × 4)	56***	3.4	22	0/6	55**	< 0		0/6	ND			
CPT-11	320 (80 × 4)	63**	3.9	22	0/6	−8	< 0		0/6	30	0.6	39	0/6
	240 (60 × 4)	39	< 0		0/6	3	< 0		0/6	ND			
	160 (40 × 4)	32	< 0		0/6	21	< 0		0/6	ND			
	80 (20 × 4)	−5	< 0		0/6	ND				ND			
VCR	3.0 (0.6 × 5)	73***	24.4	28	0/6	75***	22.2	28	0/6	5	19.6	42	0/6
	2.25 (0.45 × 5)	71***	22.9	28	0/6	73***	20.0	28	0/6	ND			
	1.5 (0.3 × 5)	62***	6.0	28	0/6	54**	2.8	28	0/6	ND			

** $P < 0.01$, *** $P < 0.001$; versus control by Dunnett's test
[a] Compounds were administered four times at 4-day intervals or daily for 5 consecutive days
[b] Maximum rate of body weight loss (< 0 indicates no body weight loss)
[c] Number of mice that died of toxicity/number of mice used

The antitumor activity of DX-8951f was further compared to that of CPT-11 using human colon, lung, breast and renal cancers. CPT-11 has been shown to exhibit high antitumor activity against human colon and lung cancers in previous preclinical [5, 14] and clinical [6, 17, 18, 20, 21] studies, and it was also effective against five of six colon cancer lines and two of five lung cancer lines in our study. DX-8951f exhibited marked activity against all lines of the above colon and lung cancers, including four lines (WiDr, PC-12, PC-6/VCR29-9 and PC-6/SN2-5S) against which CPT-11 was not effective. We have found that PC-12, PC-6/VCR29-9 and DLD-1 cells overexpress P-glycoprotein (P-gp) by Western-blotting analysis using an anti-P-gp monoclonal antibody [19]. P-gp, a plasma membrane protein, plays an important role in multidrug resistance as a drug efflux pump [2, 7, 25]. We have also shown that SK&F104864 and SN-38 (an active metabolite of CPT-11) [12, 14, 24] are significantly or moderately subject to P-gp-mediated resistance in vitro [19], as previously reported by others

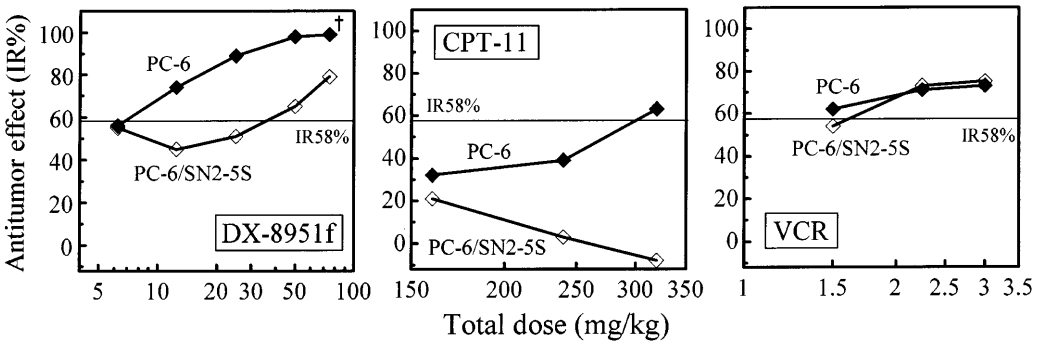

Fig. 4 Dose-effect curves for DX-8951f, CPT-11 and VCR against human lung cancers PC-6 (◆) and PC-6/SN2-5S (◇) in nude mice. Dose levels of camptothecin derivatives are expressed as those of the anhydrous free base. Values of IR% are plotted against the total dose of each compound. Data were obtained from Table 6. † indicates that some mice died of toxic effects

[1,8], but that DX-8951f is not affected by P-gp overexpression [19]. Against the above three cell lines, CPT-11 was not effective (PC-12, PC-6/VCR29-9) or exhibited antitumor activity with an IR value of 59% (DLD-1), but DX-8951f exhibited strong antitumor activity with IR values of 80% and higher. These results suggest that DX-8951f is less affected by P-gp than CPT-11 in vivo.

PC-6/SN2-5S, an SN-38-resistant variant derived from PC-6, was obtained by two courses of a stepwise selection procedure: PC-6/SN2-5 cells [10] were established from parental PC-6 cells in the first course by treatment with 0.04 to 2 ng/ml SN-38, and PC-6/SN2-5S cells were derived from PC-6/SN2-5 cells in the second course with 2 to 50 ng/ml SN-38. The mechanisms of resistance of PC-6/SN2-5 may be related to a decrease in intracellular accumulation of certain drugs, but may not be mediated by a classical efflux mechanism due to lack of expression of P-gp [10]. The mechanism of resistance of PC-6/SN2-5S has been speculated to be similar to that of PC-6/SN2-5, since the resistance patterns against various anticancer agents of these variants are similar and no expression of P-gp was detected in PC-6/SN2-5S cells. PC-6/SN2-5S cells exhibit significant resistance to

Table 8 Antitumor effects of DX-8951f and CPT-11 against human renal cancer xenografts in nude mice. A piece of tumor block was implanted into the right flank of BALB/c-nu/nu mice (day 0). The mice were randomized and divided into several groups (six mice per group) when the mean estimated tumor weight reached 134 mg on day 34. Groups of mice received i.v. injections of DX-8951f or CPT-11 every 4th day for a total of four injections (q4d × 4). Tumor weight was assessed on the 22nd day after the first administration. The tumor weight of the control group was 0.669 ± 0.055 g. The dose levels of the compounds are expressed as those of the anhydrous free base

Compound	Total dose[a] (mg/kg)	A-498			
		IR	BWLmax[b]		D/U[c]
		(%)	%	Day	
Control	0	0	< 0		0/6
DX-8951f	75 (18.75 × 4)	41	27.2	50	2/6
	50 (12.5 × 4)	28	16.6	49	0/6
	25 (6.25 × 4)	31	11.7	49	0/6
CPT-11	320 (80 × 4)	5	5.3	47	0/6
	240 (60 × 4)	32	5.0	43	0/6

[a] Compounds were administered four times at 4-day intervals
[b] Maximum rate of body weight loss (< 0 indicates no body weight loss)
[c] Number of mice that died of toxicity/number of mice used

Table 7 Antitumor effects of DX-8951f and CPT-11 against human breast cancer xenografts in nude mice. A piece of tumor block was implanted into the right flank of BALB/c-nu/nu mice (day 0). The mice were randomized and divided into several groups (5 or 6 mice/group) when the mean estimated tumor weight reached 100 to 200 mg (MX-1 day 20; Du4475 day 27). Groups of mice received i.v. injections of DX-8951f or CPT-11 every 4th day for a total of four injections (q4d × 4). Tumor weight was assessed on the 22nd day (Du4475) or the 28th day (MX-1) after the first administration. Tumor weights of control groups were as follows: MX-1 6.980 ± 1.686 g; Du4475 2.228 ± 0.395 g. Dose levels of compounds were expressed as those of the anhydrous free base

Compound	Total dose[a] (mg/kg)	MX-1				Du4475			
		IR	BWLmax[b]		D/U[c]	IR	BWLmax		D/U
		(%)	%	Day		%	%	Day	
Control	0	0	< 0		0/5	0	6.9	49	0/6
DX-8951f	75 (18.75 × 4)	100 ***	0.8	29	0/5	96 ***	6.0	37	0/6
	25 (6.25 × 4)	100 ***	1.4	21	0/5	78 ***	3.6	31	0/6
CPT-11	320 (80 × 4)	100 ***	< 0		0/5	97 ***	3.2	31	0/6
	160 (40 × 4)	98 ***	< 0		0/5	89 ***	1.4	31	0/6

*** $P < 0.001$; versus control by Dunnett's test
[a] Compounds were administered 4 times at 4-day intervals
[b] Maximum rate of body weight loss (%); with numbers in parentheses denoting the day; < 0 indicates no body weight loss
[c] Number of mice that died of toxicity / number of mice used

Table 9 Comparison of antitumor effects of DX-8951f and CPT-11 against various human tumor xenografts in nude mice. Dose levels of compounds are expressed as those of the anhydrous free base. Values are no. (%) of tumors

Cancer	No. of tumors examined	DX-8951f (total dose 75 mg/kg)		CPT-11 (total dose 320 mg/kg)	
		≥IR80%	≥IR58%	≥IR80%	≥IR58%
Gastric	2	2 (100%)	2 (100%)	1 (50%)	2 (100%)
Colon	6	6 (100%)	6 (100%)	4 (67%)	5 (83%)
Lung	5	4 (80%)	5 (100%)	1 (20%)	2 (40%)
Breast	2	2 (100%)	2 (100%)	2 (100%)	2 (100%)
Renal	1	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Total	16	14 (88%)**	15 (94%)*	8 (50%)	11 (69%)

* $P < 0.10$, ** $P < 0.05$; versus CPT-11 by Fisher's exact probability test

SN-38 (240 times that of PC-6 cells), but markedly lower resistance to DX-8951f (15 times that of PC-6 cells) in vitro (unpublished data). The present results clearly indicated, in vivo as well, that CPT-11 was not effective against PC-6/SN2-5S tumors, but that DX-8951f inhibited the growth of the resistant tumors with IR values of 58% and higher (Table 6). These findings, taken together with the antitumor effects against SC-6/CPT-11 described previously, suggest that DX-8951f may be less affected by certain types of resistance to camptothecin derivatives.

We have also examined the survival activity, as evaluated by increased life span, of DX-8951f in metastasis models of murine lung cancer cell 3LL and histiocytoma cell M5076 by i.v. transplantation, confirming that the compound is effective (unpublished results). From the potency of DX-8951f in human tumor xenograft models and murine metastasis models, the compound is expected to be clinically effective. Phase I clinical trials of DX-8951f are in progress in Europe, the United States and Japan.

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