FR901228, A NOVEL ANTITUMOR BICYCLIC DEPSIPEPTIDE PRODUCED BY *Chromobacterium violaceum* No. 968

III. ANTITUMOR ACTIVITIES ON EXPERIMENTAL TUMORS IN MICE

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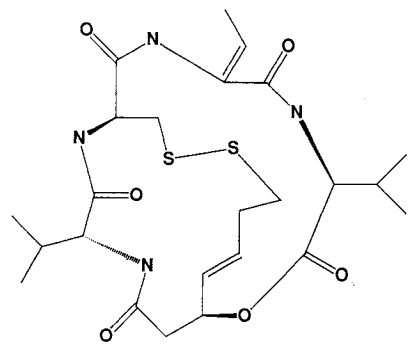
(Received for publication September 20, 1993)

The antitumor activities of FR901228, (*E*)-(1*S*,4*S*,10*S*,21*R*)-7-[(*Z*)-ethylidene]-4,21-diisopropyl-2-oxa-12,13-dithia-5,8,20,23-tetraazabicyclo[8,7,6]-tricos-16-ene-3,6,9,19,22-pentanone, isolated from *Chromobacterium violaceum* No. 968, were studied in animals. FR901228 (ip) prolonged the life of mice bearing such murine ascitic tumors as P388 and L1210 leukemias and B16 melanoma, and inhibited (iv) the growth of murine solid tumors (Colon 38 carcinoma, M5076 reticulum cell sarcoma and Meth A fibrosarcoma) and human solid tumors (Lu-65 and LC-6 lung carcinomas, and SC-6 stomach adenocarcinoma) implanted in normal and nude mice, respectively. Its antitumor activity was especially potent against murine Meth A fibrosarcoma and human SC-6 stomach adenocarcinoma which were refractory to mitomycin C or cisplatin. FR901228 also was more effective against mitomycin C-, cyclophosphamide-, vincristine- and 5-fluorouracil-resistant P388 leukemias than against non-resistant P388 in mice. These results suggest that FR901228 will be a new type of drug for the treatment of cancer.

Antitumor drugs such as mitomycin C (MMC), doxorubicin (ADR) and cisplatin (CDDP) have been widely used in the treatment of patients with various kinds of malignant tumors. These drugs effect a response by the mediation of direct inhibition of DNA synthesis in the tumor cells. However, the antitumor effects obtained in the clinic against solid tumors have been insufficient. Thus, efforts have been made to develop compounds with different modes of action which directly inhibit DNA synthesis in order to obtain adequate antitumor effects in the clinic.

Research on oncogenes is proceeding rapidly and it is expected that the inhibition of oncogene products may develop as a new stage of chemotherapy[1]. Many human tumors appear to be associated with the expression of activated *ras* genes which code the p21 protein[2]. We found that *Chromobacterium violaceum* No. 968 produced a substance (FR901228) which possesses a unique bicyclic peptide chemical structure and allows it to block the p21 protein-mediated signalling transduction pathway by reversing the morphology of Ha-*ras*-

Fig. 1. The chemical structure of FR901228.



transfected mouse NIH/3T3 (Ras-1) cells to that of the parent[3~5]. However, we also observed that FR901228 decreased mRNA expression of c-*myc*, not Ha-*ras* in the Ras-1 cells and reversed the morphology not only of Ha-*ras* but of various other oncogene-transfected mouse NIH/3T3 cells to that of the parent. These data indicate that the target of FR901228 may be a common part in several signalling pathways[3~5].

In this report we describe the antitumor effects of FR901228 against various murine and human standard tumors implanted in mice, and compare these effects with those of standard chemotherapeutic agents.

Materials and Methods

Drugs

FR901228 was prepared in our Research Laboratories; its chemical structure is shown in Fig. 1. MMC, 5-fluorouracil (5FU) and ADR were purchased from Kyowa Hakko Kogyo Co., Ltd., Tokyo, Japan; CDDP from Sigma Co., Ltd., St. Louis, MO, and cyclophosphamide (CPM) and vincristine (VCR) from Shionogi & Co., Ltd., Osaka, Japan. FR901228 was dissolved in and diluted with 10% polyoxyethylated (60 mol) hydrogenated castor oil in saline (HCO60 solution). The other drugs were dissolved in and diluted with saline. The solutions were given ip or iv at a volume of 10 ml/kg body weight. Saline or 10% HCO60 solution was given to the control animals.

In the *in vitro* culture test, all the drugs were dissolved in or diluted with culture medium supplemented with 10% fetal bovine serum (FBS) (HyClone Laboratories, Logan, UT).

Animals

Female mice of BDF_1 (C57BL/6 × DBA/2), CDF_1 (BALB/c × DBA/2), $B_6C_3F_1$ (C57BL/6 × C3H), DBA/2 and C57BL/6 strains were purchased from Charles River Japan Inc., Atsugi, Japan. Male mice of BALB/c nu/nu strain were purchased from CLEA Japan Inc., Tokyo, Japan.

Tumors (*In Vivo*)

P388 leukemia (P388) and L1210 leukemia (L1210) cells were maintained ip by serial passage in DBA/2 mice. B16 melanoma (B16) and Colon 38 carcinoma (Colon 38) were maintained sc by serial passage in C57BL/6 mice. M5076 reticulum cell sarcoma (M5076) was maintained ip by serial passage in C57BL/6 mice. Colon 26 adenocarcinoma (Colon 26) and Meth A fibrosarcoma (Meth A) were maintained sc and ip, respectively, by serial passage in BALB/c mice. The P388 resistant lines were generously supplied by Dr. M. INABA (Cancer Chemotherapy Center, Tokyo). Details of the characteristics of these cell lines have been reported[6~10]. ADR-resistant P388 leukemia (P388/ADR) was maintained ip by serial passage in CDF_1 mice. MMC-resistant P388 (P388/MMC), CPM-resistant P388 (P388/CPM), VCR-resistant P388 (P388/VCR) and 5FU-resistant P388 (P388/5FU) were maintained ip by serial passage in DBA/2 mice. Lu-65 lung large cell carcinoma (Lu-65), A549 lung adenocarcinoma (A549), LC-6-JCK lung large cell carcinoma (LC-6), MX-1 mammary adenocarcinoma (MX-1) and SC-6-JCK stomach adenocarcinoma (SC-6) were maintained sc by serial passage in BALB/c nu/nu mice.

Evaluation of Antitumor Effects on Murine Ascitic Tumors

P388 cells (1×10^6), L1210 cells (1×10^5) and P388/ADR, P388/MMC, P388/CPM, P388/VCR and P388/5FU cells (1×10^6) were inoculated ip in CDF_1 mice. B16 brei (1 : 10 brei/0.5 ml) was inoculated ip in BDF_1 mice. In the tests with P388, P388/ADR, P388/MMC, P388/CPM, P388/VCR and P388/5FU, 12 and 6 mice were used in the control and drug treated groups, respectively. In the tests with L1210 and B16, 10 mice were used in each group. Drug efficacy was assessed as a percentage of median (mean) survival time of the treated group (T) to that of the control group (C).

$$T/C\,\% = \frac{\text{Median (mean) survival time of (T)}}{\text{Median (mean) survival time of (C)}} \times 100$$

Evaluation of Antitumor Effects on Human and Murine Solid Tumors

In the experiments on murine solid tumors, fragments ($2 \times 2 \times 2$ mm) of Colon 38 and Colon 26 were implanted sc in the left flank of BDF_1 and BALB/c mice, respectively. M5076 cells (1×10^6) and Meth A cells (1×10^5) were inoculated intradermally (id) in $B_6C_3F_1$ and BALB/c mice, respectively. In all the experiments, 20 and 10 mice were used in the control and drug treated groups, respectively.

Tumor weight, as derived from caliper measurements of the length and width of tumors, was calculated by the formula: tumor weight (mg) $= 1/2 \times a \times b^2$, where a represents length and b represents width (mm).

Drug efficacy against murine solid tumors was based on the percentage of mean tumor weight of the treated group (T) to that of the control group (C).

$$\text{Growth inhibition (\%)} = \left(1 - \frac{\text{Mean tumor weight (T)}}{\text{Mean tumor weight (C)}}\right) \times 100$$

In the experiments on human solid tumors, fragments ($3 \times 3 \times 3$ mm) of Lu-65, A549, LC-6, SC-6 and MX-1 were implanted sc into the left flank of groups of 6 BALB/c nu/nu mice respectively. Initial and final tumor weights were calculated on the first injection day (just before dosing) and the last evaluation day (about 2 weeks after the first injection day), respectively. Relative mean tumor weights are shown for each group of mice.

$$\text{Relative mean tumor weight} = \text{Mean tumor weight (final)}/\text{Mean tumor weight (initial)}$$

Drug efficacy was expressed as a percent of relative mean tumor weight of the treated group (T) to that of the control group (C).

$$\text{Growth inhibition (\%)} = \left(1 - \frac{\text{Relative mean tumor weight (T)}}{\text{Relative mean tumor weight (C)}}\right) \times 100$$

Activity Criteria

The criteria for activity and toxicity in *in vivo* tumor models were estimated according to a modification of the method used by the National Cancer Institute[11]. We used two criteria as described in each table, and defined the small and large values in T/C or 1−T/C (%) as moderate (+) and good activities (++), respectively.

In Vitro Cytotoxicity Test

The MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (Sigma, Co., Ltd., St. Louis, MO) cytotoxic assay was used to measure cytotoxicity. P388 and its resistant sublines (P388/ADR, P388/MMC, and P388/5FU) were maintained and treated in suspension in RPMI 1640 medium (Nikken Bio Medical Laboratory, Kyoto, Japan) supplemented with 10% FBS (HyClone Laboratories, Logan, UT), penicillin (50 units/ml)-streptomycin (50 μg/ml) (Flow Laboratories, North Ryde, Australia), and 2-mercaptoethanol (50 μM) (Sigma, Co., Ltd., St. Louis, MO). The cells were grown in 5% CO_2-95% air atmosphere at 37°C. In the cytotoxicity assay, the cells (1×10^4) were incubated with drug in 96 well microplates (Falcon 3072, Becton Dickinson Labware, Oxnard, CA) for 48 hours, 10 μl MTT at a concentration of 5 mg/ml in phosphate-buffered saline (8 mM, pH 7.4) was added, and the cells were incubated for further 4 hours at 37°C. Formazan crystals were solubilized by adding 100 μl of 0.04 N HCl in isopropanol. The optical density of each well was measured with a Titertek Multiskan autoreader (Flow Laboratories, McLean, VA) set for absorption at 580 nm.

The percentage of cytotoxicity was calculated by the formula: cytotoxicity (%) $= (1 - OD_{580}$ in wells with drug/OD_{580} in control wells without drug) $\times 100$.

Results

Antitumor Effects on Ascitic Tumors in Mice

The antitumor effects of FR901228 on mouse ascitic P388, L1210 and B16 tumors were examined and compared with those of MMC and CDDP. The tumors were inoculated ip to mice on Day 0. The

drugs were given ip to mice once a day for 5 days (Days 1～5) in the test on P388, and once a day for 9 days (Days 1～9) in the tests on L1210 and B16. As shown in Table 1, all three drugs prolonged the life of mice bearing P388, L1210 or B16, and while FR901228 was less active than MMC or CDDP against P388 and L1210, it showed activity comparable to MMC or CDDP on B16 at a wider effective dose range (0.056～0.56 mg/kg) than either of these two drugs.

Antitumor Effects on Murine Solid Tumors in Mice

The antitumor effects of FR901228 were examined on murine solid tumors in mice. Colon 38, and Colon 26 were implanted sc, and M5076 and Meth A were implanted id on Day 0. The drugs were given iv once a day on 3 or 4 nonconsecutive days, and the tumors were weighed. The results are shown in Tables 2 and 3. When the drugs were given beginning on Day 1, FR901228 markedly inhibited the growth of Colon 38 and M5076, but not Colon 26 or Meth A (Table 2). Additionally, when the drugs were given beginning on Day 4, or on 7 or 8, FR901228 potently inhibited the growth of Colon 38, M5076 and Meth A (Table 3), and its activities against M5076 and Meth A were more potent than when it was given beginning on Day 1 (Table 2). MMC and CDDP were not active against Meth A. After iv injection of FR901228 to mice, the surface of the Meth A tumor reddened from the center and the area of necrosis darkened and gradually became wider, FR901228 slightly induced the necrosis of M5076 tumors in mice.

Antitumor Effects on Human Xenograft Tumors in Nude Mice

The experiments were performed to evaluate the antitumor effects of FR901228 on human Lu-65, A549, LC-6, SC-6 and MX-1 tumors implanted sc in BALB/c nu/nu mice. When the weights of the tumors reached about 150 mg, the drugs were given iv 3 times at 4-day intervals. Table 4 shows that FR901228

Table 1. Antitumor effects of FR901228 and the reference drugs on murine ascitic tumors in mice.

Drug	Dose (mg/kg)	P388 (ip-ip) QD[a] D1～5		L1210 (ip-ip) QD D1～9		B16 (ip-ip) QD D1～9	
		T/C (%)	Activity[b]	T/C (%)	Activity	T/C (%)	Activity
FR901228	0.032	108	−	111	−	116	−
	0.056			113	−	129	+
	0.1	121	+	118	−	126	+
	0.18			125	+	152	++
	0.32	125	+	125	+	165	++
	0.56	138	+	129	+	174	++
	1.0	83	Tox[c]	97	−	58	Tox
MMC	0.1	117	−	109	−	116	−
	0.32	138	+	118	−	139	+
	0.56	142	+	130	+	163	++
	1.0	154	+	151	++	176 (1)	++
	1.8	138 (1)[d]	+	149	+	61	Tox
	3.2	79	Tox	103	−	39	Tox
CDDP	0.32	121	+	116	−	115	−
	1.0	142	+	139	+	185	++
	3.2	>250 (4)	++	195	++	65	Tox
	5.6	83	Tox	110	−	35	Tox

[a] QD, every day.

[b] Tumor cells were inoculated ip to mice on Day 0. The drugs were given ip to mice once a day for 5 days (Days 1～5) in the P388 test and for 9 days (Days 1～9) in the L1210 and B16 tests. Criteria: +, ≧120 and ++, ≧175 for P388 and +, ≧125 and ++, ≧150 for L1210 and B16.

[c] Tox, a T/C value of <86% indicates toxicity.

[d] Number in parentheses, number of survivors on Day 30 in P388 and L1210 tests, and Day 60 in B16 test.

Table 2. Antitumor effects of FR901228 and the reference drugs on murine solid tumors in mice.

Drug	Dose (mg/kg)	Colon 38 (sc-iv) Q3D[a] Days 1, 4, 7 and 10 weight D17		Colon 26 (sc-iv) Q3D Days 1, 4 and 7 weight D14		M5076 (id-iv) Q3D Days 1, 4 and 7 weight D14		Meth A (id-iv) Q3D Days 1, 4 and 7 weight D14	
		1−T/C (%)	Activity[b]	1−T/C (%)	Activity	1−T/C (%)	Activity	1−T/C (%)	Activity
FR901228	1.0	2	−	19	−	−2	−	33	−
	1.8	25	−	30	−	28	−	39	−
	3.2	60	+	43	−	61	+		Tox
	5.6	94	++		Tox	78	+		Tox
	10						Tox		
MMC	1.0	49	−	34	−	52	−	35	−
	1.8	86	+	45	−	82	+	42	−
	3.2	97	++	83	+	93	++	71	+
	5.6		Tox[c]		Tox		Tox		Tox
CDDP	1.0	17	−			45	−		
	1.8	32	−	39	−			23	−
	3.2	41	−	74	+	86	+	46	−
	5.6	64	+	98	++	94	++		Tox
	10		Tox		Tox	100	++		Tox

[a] Q3D, every 3 days.

[b] Tumor cells were implanted sc or id to mice on Day 0. In the Colon 38 test the drugs were given iv to mice once a day on Days 1, 4, 7 and 10 and tumor weights were measured on Day 17. In the Colon 26, M5076 and Meth A tests the drugs were given iv to mice once a day on Days 1, 4 and 7 and tumor weights were measured on Day 14. Criteria: +, ≧58; ++, ≧90.

[c] Tox, a survival rate of <65% on the evaluation day indicates toxicity.

Table 3. Antitumor effects of FR901228 and the reference drugs on murine solid tumors in mice.

Drug	Dose (mg/kg)	Colon 38 (sc-iv) Q3D[a] Days 7, 10, 13 and 16 weight D17		Colon 26 (sc-iv) Q3D Days 4, 7 and 10 weight D14		M5076 (id-iv) Q3D Days 8, 11 and 14 weight D21		Meth A (id-iv) Q3D Days 7, 10 and 13 weight D21	
		1−T/C (%)	Activity[b]	1−T/C (%)	Activity	1−T/C (%)	Activity	1−T/C (%)	Activity
FR901228	0.56							46	−
	1.0	25	−	19	−	3	−	77	+
	1.8			4	−	11	−	84	+
	3.2	63	+	21	−	59	+	92	++
	5.6	76	+	39	−	85	+	95	++
	10		Tox[c]			92	++		
MMC	1.0			34	−	41	−	22	−
	1.8			45	−			41	−
	3.2			83	+	91	++	58	+
	5.6				Tox		Tox		Tox
CDDP	1.0					34	−		
	1.8			39	−			12	−
	3.2			74	+	73	+	40	−
	5.6			98	++			42	−
	10				Tox	97	++		Tox

[a] Q3D, every 3 days.

[b] Tumor cells were implanted sc or id to mice on Day 0. The drugs were given iv to mice once a day on Days 7, 10, 13 and 16 in the Colon 38 test, on Days 4, 7 and 10 in the Colon 26 test, on Days 8, 11 and 14 in the M5076 test and on Days 7, 10 and 13 in the Meth A test. Tumor weights were measured on Day 17 in the Colon 38 test, on Day 14 in the Colon 26 test, on Day 21 in the M5076 and Meth A tests. Criteria: +, ≧58; ++, ≧90.

[c] Tox, a survival rate of <65% on the evaluation day indicates toxicity.

Table 4. Antitumor effects of FR901228 and the reference drugs on human solid tumors in nude mice.

Drug	Dose (mg/kg)	Lu-65 (sc-iv) Q4D[a], 3 times weight D28		A549 (sc-iv) Q4D, 3 times weight D28		LC-6 (sc-iv) Q4D, 3 times weight D18		SC-6 (sc-iv) Q4D, 3 times weight D21		MX-1 (sc-iv) Q4D, 3 times weight D28	
		1−T/C (%)	Activity[b]	1−T/C (%)	Activity	1−T/C (%)	Activity	1−T/C (%)	Activity	1−T/C (%)	Activity
FR901228	1.8	63	−	51	−	64	−	77	−	44	−
	3.2	74	−	48	−	74	−	84	+	51	−
	5.6	83	+	70	−	82	+	92	++	63	−
	10										Tox
MMC	1.0	63	−	19	−	52	−	29	−	98	++
	1.8			33	−			48	−	99	++
	3.2	92	++	51	−	97	++	71	−	99	++
	5.6						Tox[c]	66	−		Tox
CDDP	3.2	51	−	15	−	65	−	64	−	98	++
	5.6	86	+	13	−	87	+	78	−	99	++
	10			41	−	98	++	95	++	99	++

[a] Q4D, every 4 days.

[b] Tumor cells were implanted sc to mice on Day 0. The drugs were given iv to mice three times at 4-day intervals beginning on Day 13 in the Lu-65 test, on Day 14 in the A549 test, on Day 7 in the LC-6 test, on Day 10 in the SC-6 test and on Day 14 in the MX-1 test. Tumor weight was measured on Day 28 in the Lu-65, A549 and MX-1 tests, on Day 18 in the LC-6 test and on Day 21 in the SC-6 test. Criteria: +, ≧80; ++, ≧90.

[c] Tox, survival rate of <65% on the evaluation day indicates toxicity.

inhibited the growth of Lu-65, LC-6 and SC-6 by more than 80%, and MMC and CDDP almost completely inhibited the growth of MX-1, but FR901228 did not. The three drugs were not active against A549.

Antitumor Effects on Drug-resistant P388 in Mice

Table 5 shows the antitumor effects of FR901228 on drug-resistant P388 in mice. The tumors were inoculated ip on Day 0, and the drugs were given ip once a day for 4 days (Day 1~4). The median survival times were measured. Using non-resistant P388 as a basis for comparison, FR901228 was more active against MMC-, CPM-, VCR- and 5FU-resistant P388, and was less active against ADR-resistant P388.

Cytotoxicity on *In Vitro* Cultured P388 Cells

The cytotoxic activities of FR901228, ADR, MMC and 5FU on P388 parent and three drug-resistant P388 strains are shown in Table 6. FR901228 showed strong cytotoxic effects on the non-resistant P388, with activity comparable to that of ADR and more potent than that of MMC or 5FU. It was also strongly active against P388/MMC and P388/5FU, but was much less active against P388/ADR than against the non-resistant P388.

Discussion

In the cytotoxicity experiment, FR901228 was cytotoxic to the parent P388 cells, and its IC_{50} value was about 0.37 nM (0.2 ng/ml). However, the IC_{50} value of this drug on normal human fibroblast cells was >1,000 ng/ml[3]. The concentrations of the drug required to induce 50% cytotoxicity on tumor cells were much lower than those required for a similar effect on normal human fibroblast cells. The IC_{50} values of ADR on parent P388 cells and normal human fibroblast cells were 0.35 ng/ml and 130 ng/ml, respectively. Thus, the selectivity of FR901228 for tumor cells is much greater than that of ADR. FR901228 might be an antitumor drug with weaker side effects.

Although the antitumor effect of FR901228 on B16 ascitic tumor was almost the same as that of

Table 5. Antitumor effects of FR901228 and the reference drugs on drug-resistant P388 leukemia.

Drug	Dose (mg/kg)	P388		P388/ADR		P388/MMC		P388/CPM		P388/VCR		P388/5FU	
		T/C (%)	Activity[a]	T/C (%)	Activity	T/C (%)	Activity	T/C (%)	Activity	T/C (%)	Activity	T/C (%)	Activity
FR901228	0.18	116	−	110	−	136	+	122	+	125	+	167	+
	0.32	116	−	115	−	150	+	133	+	121	+	167	+
	0.56	121	+	110	−	155	+	156	+	125	+	170	+
	1.0	126	+		Tox[b]	164	+	161	+	138	+	183	++
ADR	0.32	122	+	100	−								
	1.0	133	+	100	−								
	3.2	178	++	95	−								
MMC	1.0	122	+			109	−						
	1.8	122	+			118	−						
CPM	10	111	−					95	−				
	32	144	+					95	−				
VCR	0.1	122	+							117	−		
	0.32	133	+							113	−		
5FU	3.2	122	+									106	−
	10	144	+									94	−
	32	183	++									100	−

[a] Tumor cells were inoculated ip to mice on Day 0. The drugs were given ip to mice once a day for 4 days (Day 1~4). Criteria: +, ≧120; ++, ≧175.

[b] Tox, survival rate of <65% on the evaluation day indicates toxicity.

Table 6. Cytotoxic effects of FR901228 and the reference drugs on P388 and drug-resistant P388 leukemia cells.

Drug	IC_{50} (nM)			
	P388	P388/ADR	P388/MMC	P388/5FU
FR901228	0.37	350 (950)[a]	0.28 (0.76)	0.21 (0.57)
ADR	0.59	3,000 (5,100)	24 (41)	2.5 (4.2)
MMC	180	2,200 (12)	3,000 (17)	1,300 (7.2)
5FU	1,500	1,600 (1.1)	760 (0.51)	23,000 (15)

[a] Number in parentheses, relative resistance = IC_{50} (resistant P388)/IC_{50} (P388).

MMC and CDDP in mice, it showed weaker activities to P388 and L1210. P388, L1210 and B16 which have been used in the screening system of the National Cancer Institute[12], by which many antitumor drugs now in clinical use were found. Furthermore, FR901228 potently inhibited the growth of Colon 38, M5076 and Meth A solid tumors in mice. When FR901228 was given to mice beginning on Day 7 or 8 after Meth A and M5076 inoculation, its antitumor effects were stronger than when it was given from Day 1, and were characterized by hemorrhagic necrosis. These effects resembled those of recombinant tumor necrosis factor-α (rTNF-α), although they were somewhat weaker[13]. We reported that rTNF-α was more effective on established Meth A tumor than on newly implanted tumors in mice[14], and that its antitumor effects may be derived from an indirect mechanism related to the growth of the implanted tumors rather than to its direct cytotoxicity[13,14]. Thus, our findings suggest that indirect mechanisms as well as direct cytotoxicity have a role in the antitumor effects of FR901228. The antitumor spectrum of FR901228 may therefore be different from that of many antitumor drugs in clinical use, and FR901228 may be clinically effective on solid tumors which are refractory to many antitumor drugs.

Moreover, FR901228 showed strong antitumor effects on human tumors such as Lu-65, LC-6 and SC-6 in nude mice. Thus, these effects might also be expected in humans.

In *in vitro* and *in vivo* experiments, FR901228 showed cross-resistant to ADR. P388/ADR is reported to be a MDR leukemia cell line which expresses *p*-glycoprotein[10]. Because of its cyclic peptide structure and its lipophilicity, FR901228 may be a substrate for *p*-glycoprotein in MDR cells.

In mice FR901228 showed strong antitumor activities against MMC-, CPM-, VCR- and 5FU-resistant P388, whereas it showed cross-resistant to ADR. The *in vitro* cytotoxic activities of FR901228 on MMC- and 5FU-resistant P388 cells were similar to those on the non-resistant cells, but were weaker on ADR-resistant P388. We have no explanation for this, and can only speculate that FR901228 has a different mode of action from MMC, CPM, VCR and 5FU.

In summary, FR901228 is a new type of antitumor drug and its antitumor spectrum may be different from that of MMC or CDDP in the clinic.

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